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PRIMARY MELANOSARCOMA OF THE LEPTOMENINGES *

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Melanomas are malignant tumors arising in those regions where melanin-bearing cells are present. The usual sites are the pigmented nevi in the skin, the uveal tract of the eye, the pigmented layer of the retina, the adrenal gland, various pigmented regions of the mucous membranes and the meninges of the brain.

Primary melanoma of the meninges is considered to be rare and only 29 cases have been reported. A review of the literature reveals cases reported by the following authors: Virchow,[1] Sternberg,[2] Stoerk,[3] Hirschberg,[4] Pick,[5] Minelli,[6] Boit,[7] Thorel,[8] Bösch,[9] Lindbom,[10] Lua,[11] Hesse,[12] Esser,[13] Schopper,[14] Koelichen,[15] Kiel,[16] Matzdorff,[17] Neubürger,[18] Omodei-Zorini,[19] Schmid,[20] Baumecker,[21] Dieckmann,[22] Farnell and Globus,[23] Foot and Zeek[24] (2 cases), Heilmann,[25] de Blasi,[26] Lackerbauer,[27] Garcin and associates,[28] and Jacob.[29] In a review of the reported cases Weimann[30] found that only 4 could be considered definitely to be primary melanosarcoma of the brain. Omodei-Zorini[19] in his critical study of 20 cases could find only 12 certain cases.

The case reported here showed a diffuse melanosarcomatosis arising primarily in the pia with secondary involvement of the brain parenchyma. The histological study of the process in the leptomeninges and the brain revealed that the various cells of the neoplasm belonged to various developmental stages in the formation of the chromatophore.

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Report of Case

Clinical History: On May 30, 1932, a white male, aged 82 years, was admitted to the Strong Memorial Hospital in a comatose condition. According to his family, the patient had enjoyed excellent health except that for the past few months he had been subject to dizzy spells. Thirty-six hours before admission he complained of feeling ill, a few minutes later he lost consciousness and had a clonic convulsion involving the left arm and leg.

Physical Examination: The patient was a well developed, poorly nourished, elderly man in a comatose state. The hands and lips were cyanotic, and the skin was cold and clammy. The respirations were stertorous, shallow and rapid. The temperature was 40.7° C. by rectum, the pulse 58 and respirations 52.

Neurological examination disclosed a conjugate deviation of the eyes to the left and the pupils contracted and fixed to light. The fundi showed arteriosclerosis, but no blurring of the optic discs. The extremities were flaccid and the deep tendon reflexes were absent bilaterally. Plantar stimulation resulted in dorsal flexion of the large toe on the left side and there was a positive Hoffmann's sign on the left side.

Course of Illness: The patient did not regain consciousness and died 1 hour after admission.

Clinical Diagnosis: Intracranial hemorrhage in the right internal capsule.

Autopsy Report

The autopsy was performed 14 hours after death. Except for the brain, no abnormal pigmentations over the body or in the internal organs were found. Examination of internal organs showed generalized arteriosclerosis and bronchopneumonia.

The brain weighed 1500 gm. and was of normal consistence with moderate widening of the sulci over the frontal lobes. There was a moderate thickening of the larger cerebral arteries and the blood vessels in the Rolandic fissure were greatly engorged on the right side.

The remarkable feature was the extreme brownish black pigmentation which was symmetrically but unevenly distributed in the leptomeninges on both sides of the brain. The pigment was most intense around the Sylvian and Rolandic fissures, extending far out over the frontal and parietal lobes but diminishing as the frontal and occipital poles were reached. On the mesial surface and base of the brain the pigmentation was much less marked. As the pigmentation diminished in amount it was arranged in discrete flecks of various sizes measuring from 0.5 to 5 mm. in diameter (Fig. 1). Where the meninges were stripped from the underlying brain tissue a few small gray to black dots measuring up to 2 mm. were seen to invade the

cortex. After fixation in formalin careful sectioning disclosed in all only four small, dark, circumscribed nodules extending from the meninges into the cortex of the cerebrum. These nodules were found in the pars opercularis of the gyrus frontalis inferior, the pars superior of the gyrus frontalis medius (two nodules) and the area parolfactorius of the right side. Macroscopically this invasion was limited to the gray matter and did not extend into the medullary layer.

The ventricular surfaces were free of pigment with the exception of the right wall in the recessus suprapinealis and along the tela choroidea ventricularis where the melanosis extended up to the foramen of Monro, and a few small discrete flecks in the right lateral ventricle.

The spinal cord was not removed except for a small portion of the upper cervical cord.

Microscopic Examination

Pieces of tissue were taken from various parts of the central nervous system, including especially those regions in which the brain parenchyma was invaded by the neoplastic process. The stains employed were thionin, hematoxylin-eosin, Van Gieson, Globus' modification of Cajal's method for astrocytes, Penfield's modification of Hortega's method for microglia and oligodendroglia, Weil's myelin stain, Herxheimer's scharlach R stain for fat, Marchi's stain, and Perdrau's stain for connective tissue. The pia over the gyri and in the sulci was carefully stripped off and various silver methods were employed to stain the nerve endings. The most satisfactory method was found to be Snessarew's modification of Cajal's technique.[31]

The pigment showed the reactions characteristic of melanin. The granules were completely decolorized by hydrogen peroxide, nascent chlorine and 2 per cent chromic acid. Similarly, treatment of sections with 0.5 per cent potassium permanganate for 24 hours, followed by 0.5 per cent oxalic acid, resulted in complete bleaching of the melanin granules. Concentrated solutions of acids and alkalis and the ordinary fat solvents did not dissolve the granules. The pigment gave no reaction with scharlach R and tests for iron were negative. In unstained sections the granules were light to dark brown in color; with thionin the granules were dark green; in hema-

toxylin-eosin and Van Gieson stains the granules were light to dark brown, and with silver stains the granules were black. The silver stains also brought out the granules in greater abundance in all types of pigment cells.

Meninges: The dura was free of melanin-containing cells. The leptomeninges were as a rule not thickened and no inflammatory cells were seen. In every section that contained meninges chromatophores were found (Fig. 2). Most frequently the chromatophores were in single layers and limited to the pia intima and the pial lymph space about the blood vessels. However, in those regions where the pigment flecks were present the chromatophores were sometimes several layers in thickness. In such areas it was not infrequent to find that these pigmented cells had invaded the brain parenchyma by way of the perivascular spaces (Fig. 3). The arachnoid contained chromatophores only in those regions where the pigment flecks were large and active proliferation of cells was apparently taking place.

In the strips of pia the pigmentation was found to be variable. Those areas that were relatively unpigmented showed typical chromatophores, isolated or arranged in groups. In the latter the processes of individual cells appeared to anastomose freely, producing a so-called chromatophore net. In general, the melanin granules were usually found within cells, but occasionally pigment was seen lying free in the pia. The chromatophores usually had a very definite relation to the blood vessels, as may be seen in Figure 4. In this illustration the round pigment cells are arranged around the walls of the blood vessels. These cells are limited to the membrane forming the pial lymph space but do not invade the adventitia. In other sections the chromatophores were arranged in such a manner that their processes ran parallel with the course of the blood vessels.

The pigment flecks showed, under low magnification, a dark center with radiating processes going off from the core very much like the spokes of a wheel (Fig. 5). Under higher magnification the center was found to consist of numerous small, round pigmented cells. The radiations were made up of several different types of pigment cells (Fig. 6). Consequently, five types of pigment cells could be differentiated as follows:

(1) Round cells which measured 8 to 12 μ in diameter. These cells were most numerous and were found usually in the center of

the pigment fleck. The melanin was in the form of minute granules which were of a light brown color in unstained sections. The nucleus was vacuolated and centrally placed; occasionally double nuclei were seen.

(2) Oval cells with and without short plump processes. The cell body had an average size of 15 by 10 μ. These cells were fairly numerous and were found most frequently in the immediate periphery of the core and occasionally within the core. The granules were identical with those found in the round cells. The nuclei were vacuolated and centrally placed. These cells were apparently transitional forms between the round pigment cells and the spindle-shaped chromatophores with long processes (Fig. 7).

(3) Spindle-shaped chromatophores. The average size of the cell body was 30 by 10 μ. These cells formed the most numerous proportion of the chromatophores and usually possessed two long delicate processes which sometimes attained a length of 200 μ. They were found in the periphery of the pigment fleck and the relatively unpigmented areas. The granules were fairly numerous and of a darker brown color than those seen in the round or oval cells. The nucleus was round and vacuolated. Occasionally, giant types were seen whose cell body measured 70 by 30 μ and whose processes were 300 μ in length. In these cells double and triple nuclei were frequently found.

(4) Polyhedral cells with and without short plump processes. The average size of the cell body was 15 by 12 μ. These cells were few in number and were found in those areas where the oval cells with short processes occurred. The melanin granules were identical with those found in the round cells. The nucleus was vacuolated and centrally placed. Much larger cells containing two or more nuclei were rarely seen. The general impression obtained was that these cells were transitional forms between the round cells and the typical stellate-shaped chromatophores (Fig. 8).

(5) Stellate-shaped chromatophores. The average diameter of the cell body was 20 μ. These cells were relatively few in number. As in the case of the mature spindle-shaped chromatophores, these cells were found in the periphery of the pigmented flecks and in the relatively unpigmented areas. They contained various numbers of processes, the usual number being four. The processes were long and delicate, and sometimes reached a length of 150 μ. The granules

were similar to those found in the spindle-shaped chromatophores. The nuclei were round and vacuolated and usually centrally placed. Giant stellate cells were rarely seen. The body of these cells was very large with a diameter of 50 μ, and the processes occasionally reached a length of 200 μ. Double nuclei were infrequently seen.

The pigment flecks in the walls of the third and right lateral ventricle were similar histologically to those found in the pia.

Nerve Endings of Pia: Numerous strips of pia taken from various regions were stained for nerve endings in order to study the possible relation between them and the pigment flecks. None was discovered. The most frequent type of nerve ending seen is illustrated in Figure 9.

Parenchyma: The nerve cells appeared to be fairly well preserved in thionin-stained sections, but in scharlach R fat stains numerous small fat globules were seen in the cytoplasm. This "lipoid degeneration" was found throughout the entire cortex but was most marked about those regions where the neoplastic process had extended into the brain parenchyma. In the periphery of the invading tumors the nerve cells contained melanin granules in various amounts. In the frontal and the temporal lobes there was a moderate degree of sclerotic change in the nerve cells of the third layer. In the hippocampus numerous small, acellular areas were found, the so-called "Verödungsherde." The nerve cells in the lenticular nucleus and thalamus contained moderate amounts of lipochrome. The Purkinje cells of the cerebellum were well preserved and free of lipochrome pigment. The cells of the substantia nigra and locus cæruleus appeared normal and contained normal amounts of large melanin granules.

The astrocytes were not hypertrophied or hyperplastic. In those regions where the neoplastic process had invaded the parenchyma the protoplasmic and fibrillary astrocytes contained melanin granules within their cytoplasm and in their processes. The oligodendroglia were free of pigment granules. The microglia showed various stages of pathological change. Some appeared normal, others contained occasional melanin granules in their processes, while others were so completely filled with melanin pigment that they were globular in form and looked very much like the round pigmented cells found in the pigment flecks of the pia and in the tumor nodules of the parenchyma. The pigment cells could be differentiated from

the "Gitterzellen" by the fact that the former contained a vacuolated nucleus and the cytoplasm was free of fat. In the brain substance the blood vessels were increased in number and engorged about those regions where the tumor had invaded the parenchyma. The arteries showed moderate arteriosclerosis and calcification of the blood vessel walls occurred in the hippocampus and the lenticular nuclei. Occasionally, minute melanin granules were found in the endothelial cells of the capillaries. No melanin-containing cells were found within the lumen of the blood vessels.

Stains with Marchi and scharlach R showed degeneration in the medullary layers where the cortex was involved by the tumor. No degeneration was found in the pyramidal tracts with myelin or fat stains.

The cranial nerves were intact. The pia encircling the nerves was frequently outlined by chromatophores, but no infiltration into the epineurium was observed.

Tumor in the Parenchyma: The involvement of the parenchyma by the neoplastic process was minimal in contrast to the diffuseness of the process in the meninges. Microscopic study revealed many more areas of invasion of the cortex than could be seen macroscopically. These nodules were deeply pigmented and in spite of much search no unpigmented nodules were found.

In view of the fact that all the nodules were identical in structure, a description of the largest one is deemed sufficient (Fig. 10). The infiltrative nature of the neoplasm by way of the perivascular spaces is well illustrated and the process is limited to the cortex. However, the perivascular manner of distribution cannot explain the whole means by which this invasion occurred since pigment cells were found lying free in the brain parenchyma in the peripheral regions of the tumor (Fig. 11). Apparently the pia-glia membrane was not an impenetrable barrier against the tumor cells, for frequently a heavily pigmented cell with a round vacuolated nucleus was found in the act of passing through the membrane, half of the cell being in the perivascular space and the other half in the parenchyma. Occasionally, isolated spindle-shaped chromatophores were found lying free in the parenchyma in no close proximity to blood vessels whose perivascular spaces were filled with melanin cells.

The general arrangement of the pigmented cells in the tumor suggested a typical melanosarcoma. The spindle-shaped cells were

arranged in bundles going in all directions, so that transverse, longitudinal and oblique aspects of these sheaves of cells could be seen. Not infrequently whorls of compact masses of spindle cells were observed (Fig. 11). Among these bundles of pigmented spindle cells were found round, oval and polygonal pigmented cells. In the more peripheral regions of the tumor the pigment cells formed a ring several layers thick about the blood vessel and definite invasion of the adventitia by the chromatophores occurred (Fig. 11). In teased preparations from the nodule all the types of pigment cells found in the pia were seen (Fig. 12).

With connective tissue stains, such as Van Gieson's and Perdrau's, typical reticulum and collagen fibers were found in large numbers throughout the tumor (Fig. 13).

Discussion

Occurrence of Chromatophores in the Meninges

Chromatophores in the leptomeninges were, according to Virchow,[1] first described by Valentin. Since then Kölliker,[32] Obersteiner,[33] Charpy,[34] and others have described the occurrence of these chromatophores in the meninges of normal adult brains. Obersteiner [33] described these cells in the adventitia of the blood vessels and pia of the medulla oblongata and could trace these chromatophores in the pia around the blood vessels into the brain substance. Kölliker [32] and Golman [35] insisted that the pigmentation was limited to the pia. The usual sites of these chromatophores are in order of frequency: base of the medulla oblongata and cerebellum, about the dorsal surface of the upper cervical region, base of the cerebral peduncles, region of the optic chiasma, and along the sylvian fissure and the inferior surfaces of the frontal, temporal and occipital lobes.

Regarding the appearance of chromatophores of the pia in relation to age, Broniatowski [36] studied the pia mater about the medulla oblongata in a series of patients from the ages of 3 to 71 years. He found that melanin-containing cells appear at the age of 9 years and that after puberty they do not increase in number but that the color of the granules changes from a light brown to a dark brown. Farnell and Globus [23] state that they have frequently seen chromatophores in infants and have found them in a fetus of 5½ months. The relation of pigmentation of the pia to race has been mentioned

infrequently. Mohnike [37] noted the presence of pigment in the leptomeninges of the brain in the Javanese, but did not find it in the negro. According to Freeman,[38] chromatophores are more numerous in the brains of negroes than in those of the white race. Virchow stated that pigmentation of the base of the brain was present in all normal adults of the Caucasian race. Symmers [39] reported excessive pigmentation of the pia in more than half of a series of 177 routine autopsies on Egyptians.

The chromatophores of the pia can become hyperplastic, leading to a diffuse melanosis of the pia, and the pigment flecks are often symmetrically arranged on both sides of the brain. In the gray horse (Virchow), calf (Casper), and sheep (Schwalbe and Weidenreich), excessive pigmentation of the meninges is not uncommon and is usually associated with abnormal pigmentation of the skin. According to Dawson,[40] melanomas of the meninges have been described in the horse and sheep. In man, Rokitansky,[41] Oberndorfer,[42] Grahl,[43] Maclachlan,[44] and Berblinger [45] have described cases of marked pigmentation of the brain allied with numerous pigmented nevi of the skin. Similarly, in the cases of primary melanoma of the meninges reported by Esser,[13] Lua,[11] Lindbom,[10] and Schopper,[14] numerous nevi in the skin were present. The presence of developmental disturbances in cases of abnormal melanotic pigmentation and melanoma of the meninges was reported by Hamill and Rothstein [46] (syringomyelia), Koelichen [15] (syringomyelia), Bösch [9] (multiple sclerosis), and Berblinger [45] (glioma).

According to Spielmeyer,[47] under various chronic pathological conditions and in senility the number of chromatophores increases. General paresis is the condition most apt to produce this increase and in chronic encephalitis involving the cells of the substantia nigra the amount of melanin may be greatly increased in the pia about the cerebral peduncles. Jakob [48] states that in any chronic irritative condition of the pia a marked hypertrophy and hyperplasia of chromatophores may occur. This excessive pigment is found in the pia, the fibrous neuroglia and the microglia. In the glia cells the pigment is in the processes in the form of large granules, but in the microglia this pigment is finely granular. The ganglion cells and oligodendroglia cells are free of this pigment. As a possible explanation of this hyperpigmentation the observation of Peck [49] may be utilized. He has shown experimentally that the increased pigmen-

tation in the skin associated with inflammation is related to a sudden and strong dopa reaction, even in the absence of light.

It is a well known fact that hyperpigmentation of the skin occurs in Addison's disease. Similarly, Fagge [50] finds that the pigmentation of the pia may be greatly increased in this disease also. This may be explained in the same manner that Bloch [51] explains this condition in the skin. Due to the failure of the adrenal glands to synthesize adrenalin from its mother substance, an excess of this normal precursor circulates in the blood. This precursor is closely related to 3–4 dihydroxyphenylalanine (dopa), possibly identical with it. Circulating in the blood, it is possible that the excess of this adrenalin precursor on reaching cells in the pia is acted on by the contained melanin-forming ferment (dopa oxydase) and oxidized into melanin.

Origin and Nature of the Chromatophore

Active debate has arisen regarding the origin and nature of the chromatophore. In the lower animal kingdom most authorities look upon the chromatophore as a specialized cell which is derived originally from the mesoblast and which retains an intimate relation with the nervous system (Ballowitz,[52] and Königs [53]). Stockard [54] believes that in batrachians these cells arise from the mesoblastic tissue in the ovum and embryo and remain distinct throughout the life of the animal. In man and the higher mammals Bloch [51] distinguishes between the chromatophores and the melanoblasts. According to him, the melanoblast is capable of forming melanin pigment and is derived from ectodermal and mesodermal tissue. The ectodermal melanoblast is found in the basal layer of the epidermis, the follicles and matrix of pigmented hairs, in pigmented nevi, in the retina, and is probably identical with the dendritic cells found in the skin and mucous membranes of ectodermal origin. The mesodermal melanoblasts are found in the uveal tract of the eye, in the meninges and in the Mongolian spots or blue nevi. The chromatophore is, according to Bloch, a mesodermal cell merely containing melanin pigment which it has phagocytozed, but is itself incapable of forming pigment.

Bloch's theory of the chromatophore in the skin can be reconciled with the epithelial school of adherents who believe that the melanin is produced in the basal cells of the epidermis and is discharged and taken up or phagocytozed by mesodermal cells in the

dermis to form the chromatophore. In the meninges, however, this conception is difficult to accept unless we introduce the presence of epidermal embryonic rests or consider the melanin to arise from the cells of the substantia nigra, locus cæruleus, and so on. It is a well known fact, as mentioned previously, that the number of chromatophores around the cerebral peduncles may be increased in chronic encephalitis where the cells of the substantia nigra are affected. Dieckmann [22] observed nevi in the pia mater and believed that they gave rise to the pigmented flecks in the meninges. According to him these nevi contain, besides spindle-shaped chromatophores, round and polygonal cells which may contain various quantities of melanotic pigment or may be completely free of pigment. Consequently, it is possible that these nevi may produce chromatophores, as Ewing [55] has demonstrated in the deep pigmented nevi of the skin. Ewing, however, cautions against too general an application of this origin of the chromatophore and believes that the adult chromatophores in the meninges have no connection with nevus cells.

Recently an increasing collection of evidence suggests the possibility that melanin production is always of neurectodermal origin. Weidenreich [56] expressed this idea when he said that "the primordial pigment cells arise from a detached portion of the neural tube." In the past the leptomeninges have been considered to be entirely of mesodermal origin as a result of the work of His,[57] von Kölliker,[58] and Weed.[59] The work of Oberling,[60] and Harvey and Burr,[61] however, suggests that neurectodermal elements contribute to the formation of the meninges. This would suggest that neurectodermal tissue might form the pigment which would then be phagocytozed by mesodermal cells to form chromatophores.

Ribbert [62] believes that the rigid distinction between chromatophore and melanoblast does not exist because under certain conditions the chromatophore can become active in the formation of melanin. He considers the chromatophore as a specially characterized cell of mesodermal origin. In the choroid of the eye, Miescher [63] observed that the dopa reaction is positive during a short period of embryonic life, coincident with the formation of pigment by the mesodermal melanoblasts, and that after birth these cells become dopa-negative. In other words, the chromatophores are mesodermal melanoblasts in a resting state. Under pathological conditions they

may resume their embryonic activity. Similarly, Bloch and his coworkers have shown that the melanin-containing cells in the meninges are dopa-positive in embryonic life but lose this reaction later. In all probability, therefore, the melanin in the chromatophores of the meninges is an autochthonous product and in the formative stages these chromatophores are true melanoblasts. Such an interpretation would agree with Stockard's observations.

Origin of Melanomas

The origin of melanomas is still in a highly controversial state and presents many perplexing problems. Dawson [40] has very ably reviewed this subject in his monograph. The school of Unna claims their origin from the epidermis. In recent years the theory of the nervous origin of nevi and melanomas has gained adherents. The mesodermal school is divided. Simon considers melanomas to arise from young undifferentiated cell forms; von Recklinghausen believes they arise from a proliferation of the endothelium of lymphatic vessels; Pick and Jadassohn believe they spring from the endothelium and perithelium of blood vessels; and Ribbert [62] believes that melanomas arise from the chromatophores and consequently calls these neoplasms chromatophoromas.

The school believing in the epidermal or epithelial origin claims that the melanomas in the meninges arise from displaced embryonic ectodermal rests. Wieting and Hamdi [64] differentiated between two types of pigmented tumors: the chromatophoroma consisting of cells which phagocytoze melanin secondarily, and melanoblastoma composed of cells which produce melanin. This school consequently considers all melanoblastomas to be of epithelial origin and many pupils of the ectodermal school would call these tumors melanocarcinomas, insisting that the chromatophoromas arise from cells which are capable only of taking in melanin pigment. Bloch [51] does not see the need of such a distinction. According to him a melanocarcinoma arises from ectodermal melanoblasts and a melanosarcoma arises from mesodermal melanoblasts.

The nervous theory has been championed by Ewing.[55] Such a theory would explain very well the melanomas arising primarily in the adrenal gland since the chromaffin cells of the suprarenal medulla are of sympathetic origin and arise from the neurectoderm which gives rise to the ganglion cells and the sheath of Schwann cells.

Similarly, the findings of Oberling [60] and Harvey and Burr [61] would suggest that a neurectodermal origin of melanoma in the meninges is possible. In 1882 von Recklinghausen [65] discussed the origin of nevi of the skin in relation to multiple fibromas of the skin and 17 years later Soldan [66] confirmed his findings and concluded that nevi were a phase of neurofibromatosis. He believed the nevus was nervous in origin since he found nerve fibrils among the nevus cells. Laidlaw and Murray [67] have proposed an ingenious hypothesis that the pigmented mole is related to the tactile spots of reptiles and amphibia. In his masterly work Masson [68] rediscovered Soldan's findings and elaborated upon them in detail. He found that the nevus cells were derived from specialized cells in the sensory nerve endings of the skin. The superficial pigmented nevus which occurs in the epidermis of the skin is derived from the epithelial-like cells of the Merkel-Ranvier body and the chromatophores or cells of Langerhans. These latter cells lie among the epithelial cells accompanying the neurofibrils and are capable of producing pigment. (Bloch, however, claims that these Langerhans cells are not melanoblasts but nerve cells.) Should this type of nevus undergo malignant changes it would be a melanocarcinoma. The deep pigmented nevus occurring in the dermis arises from the specialized cells in the core of the Meissner corpuscle. As in the case of the specialized cells of the Merkel-Ranvier body, the origin of these cells is in doubt, although most authorities who have worked on this subject believe these cells are mesodermal in origin. Consequently, a malignant neoplasm arising from a Meissner corpuscle would give rise to a melanosarcoma.

Stöhr [69] described occasional nerve endings in the pia which are very similar morphologically to the Meissner corpuscles found in the dermis. The close relation between the chromatophore and the nerve endings in the pia mater of man has been noted by Golman,[35] and Snessarew.[70] Dieckmann,[22] and others, found nevi in the pia mater which bore a close resemblance to those seen in the skin. In each of the 2 cases of melanoma of the meninges reported by Foot and Zeek [24] they were able to find fibrils "in some way connected with peripheral nerves" in the tumors. Their findings led them to believe that melanomas are derived from the nerve endings in the pia.

Consequently, in the present study, strips of pia were stained for nerve endings in order to study the relation between the pigment

flecks and the nerve endings. No specific relation could be discovered, yet this does not preclude the possibility that the originally malignant pigment flecks might have arisen from a Meissner-like corpuscle in the pia.

The exact place of origin of the malignant neoplasm could not be found, as is usually noted in the cases reported in the literature. This may be understood on the basis that the meningeal involvement is apt to be diffuse as a result of the ease with which tumor cells can be disseminated over the brain by the constant flow of the cerebrospinal fluid in the subarachnoid space. It is not difficult to understand, therefore, that a diffuse melanosarcomatous infiltration of the meninges can occur without involvement of the cerebral parenchyma, as seen in the cases of Virchow,[1] Stoerk,[3] Thorel,[8] and Esser.[13] The general impression of pathologists regarding diffuse tumors in the meninges and brain parenchyma is first that the primary seat of the tumor is in the meninges and secondarily that it invades the nervous parenchyma, as seen in the cases of Schopper,[14] Sternberg,[2] Bösch,[9] Matzdorff,[17] and Lackerbauer.[27] I believe that the case reported here is similar since the involvement of the meninges was much greater than that of the parenchyma.

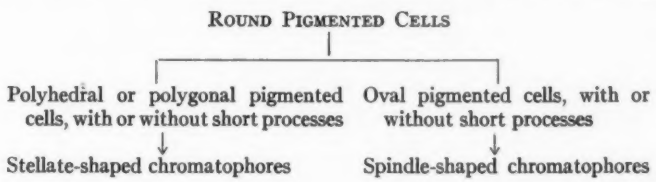
In view of the rather general belief that the meninges are of mesodermal origin, the followers of the mesodermal school have felt that the occurrence of primary melanoma in the meninges was a proof of their theory. Baumecker [21] interpreted his findings as evidence that melanomas arise from the endothelial cells of the blood vessels. Many workers have noted the relation of pigmented cells to the blood vessels in primary melanoma of the brain, and Minelli [6] states that such a tumor should be called a melanotic perithelioma. It is now generally accepted that there are no true lymphatic vessels in the brain and consequently the theory of von Recklinghausen could not be applied to primary melanoma of the brain.

The views of Ribbert [62] have been most popular in interpreting the origin of melanoma in the brain. Virchow [1] found all types of transition from simple hyperplasia and hypertrophy of pigment cells to sarcomatous changes, and believed that the widespread melanosis was derived from the hyperplasia and hypertrophy of the chromatophores. The presence of unpigmented cells so often seen in melanomas is interpreted by Ribbert and Matzdorff [17] as immature chromatophores. Hirschberg,[4] Pick,[5] Koelichen,[15] Schmid,[20] Esser,[13] and others believe melanomas arise from the chromatophores nor-

mally present in the meninges. In the cases of Boit [7] and Lindbom,[10] the tumors arose primarily in the dura mater and in view of the fact that chromatophores are never normally found in the dura, Boit believed that the chromatophoroma in his case originated from displaced chromatophores.

Summary

A review of the literature of the various hypotheses regarding the origin and nature of the chromatophore of the leptomeninges leads me to conclude that chromatophores can become active in the production of melanin and give rise to melanoma. This is in agreement with Ribbert's view that no rigid distinction between melanoblast and chromatophore is possible. The chromatophores of the leptomeninges are very much like those found in the choroid of the eye; they are mesodermal melanoblasts in a resting state. In other words, the melanin in the chromatophores of the meninges is an autochthonous product and not the phagocytosed pigment produced by other cells. Under certain pathological conditions these chromatophores or dormant melanoblasts can resume their embryonic activity in the production of melanin and behave like true melanoblasts. From the present study similar conclusions can be drawn. The chromatophores normally present in the pia can take on the rôle of active melanin production. The formation of the chromatophore from an undifferentiated round pigment cell is suggested from a study of the structure of the pigmented flecks found in the leptomeninges. In the center of the fleck numerous, small, round pigmented cells were found. The periphery of this core consisted of polymorphous pigmented cells. In the most peripheral portions typical stellate and spindle-shaped chromatophores were found. Thus, the polymorphism of the pigmented cells in the tumor can be considered to be due to the fact that the various cells are merely different transitional forms in the development of the chromatophore. This development can be illustrated in the following way:



Matzdorff [17] concluded that the round pigmented cell was an incomplete development of the chromatophore.

Similarly, it is apparent that the chromatophore in the pia, under certain pathological conditions, can take on malignant characteristics and give rise to a melanosarcoma. This is, I believe, what happened in the case here reported. One may call this tumor a chromatophoroma or a melanoblastoma. The general appearance of the tumor nodules is strongly suggestive of melanosarcoma.

No evidence could be found suggesting that the neoplasm may have arisen from nerve endings in the pia. The close relation between the chromatophores and the nerve endings in the pia mater of man normally is interesting. It is possible that the malignant condition arose from a Meissner-like corpuscle in the pia, but no evidence of such a phenomenon could be found in this case.

Conclusions

1. Chromatophores normally present in the pia can take on melanoblastic functions. It is suggested that the chromatophore is a resting mesodermal melanoblast.

2. Under pathological conditions, as in malignancy, the chromatophore can resume its melanoblastic activity and give rise to a primary melanosarcoma of the leptomeninges. This is, I believe, what occurred in the case reported here.

3. The development of typical stellate-shaped and spindle-shaped chromatophores from round pigment cells is suggested by study of the pigmented flecks in the pia.

4. The parenchymatous involvement was secondary and occurred by way of the perivascular spaces.

5. The various types of pigmented cells seen in the tumor nodules of the parenchyma were identical with those found in the pigmented flecks of the pia.

Note. I am grateful to Drs. George H. Whipple, William S. McCann, William B. Hawkins and Wilbur K. Smith for valuable advice. I also wish to express my great appreciation to Dr. George W. Corner, who kindly allowed me laboratory facilities.

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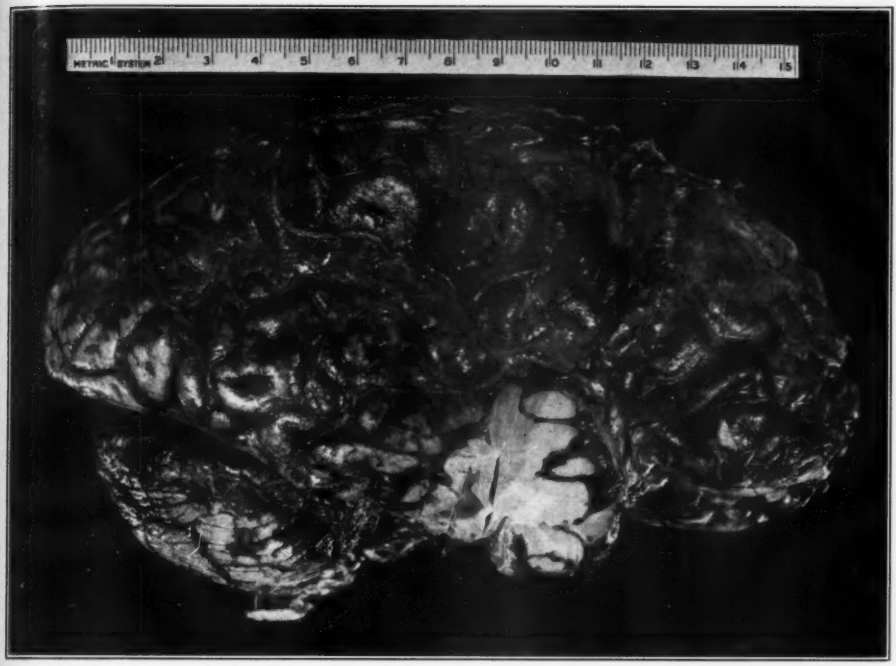
DESCRIPTION OF PLATES

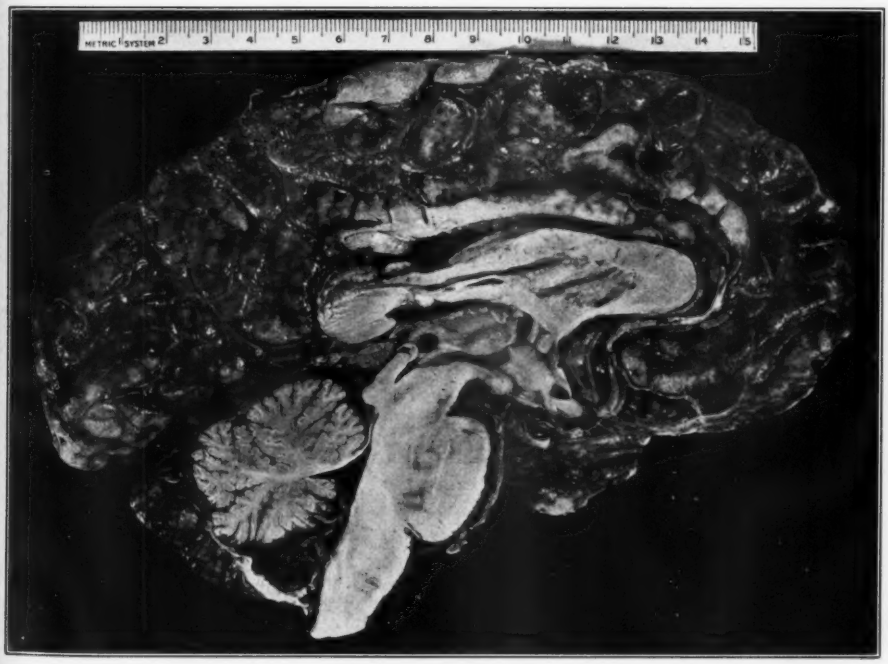
PLATE 75

FIG. 1A. Lateral view of brain.

FIG. 1B. Mesial view of brain.



1A



1B

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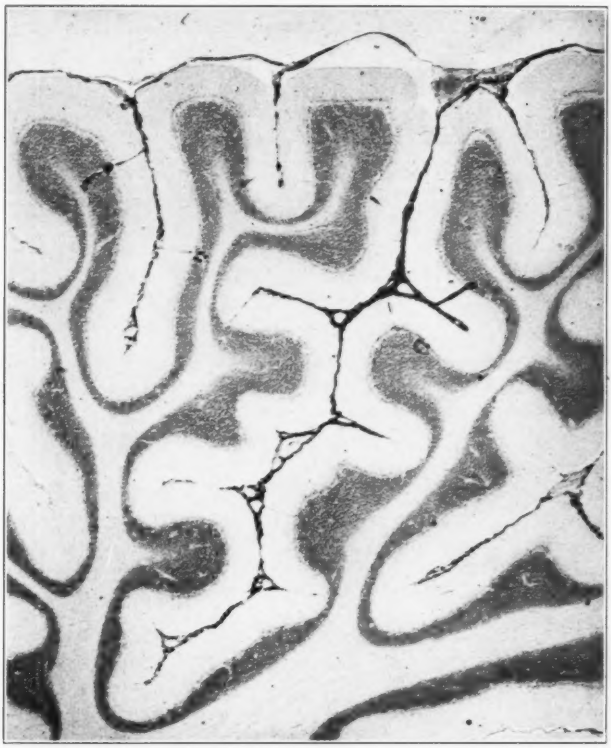
Plate 76

Fig. 2. The pia is diffusely involved by the melanotic process. The blood vessels entering the cortex are outlined by the melanin cells. These are within the perivascular spaces. Note the lack of pigmentation in the medullary layer. Thionin stain. × 15.

Fig. 3. Marked infiltration of the meninges with pigment tumor cells. The invasion of the parenchyma by way of the perivascular spaces is well shown. In the cortex the perivascular spaces are filled with pigment cells. Van Gieson's stain. × 50.

Fig. 4. Strip of pia showing the relation of the pigmented cells to the blood vessels. Thionin stain. × 100.



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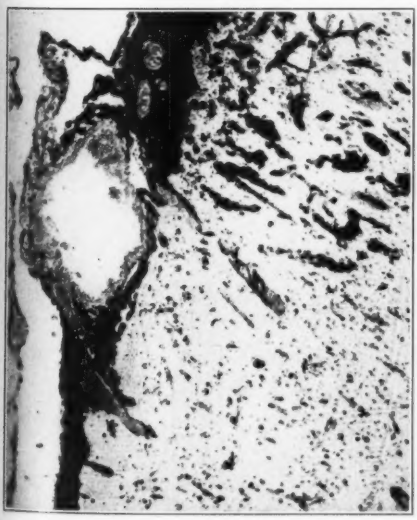

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4

Akelaitis

Primary Melanosarcoma of the Leptomeninges

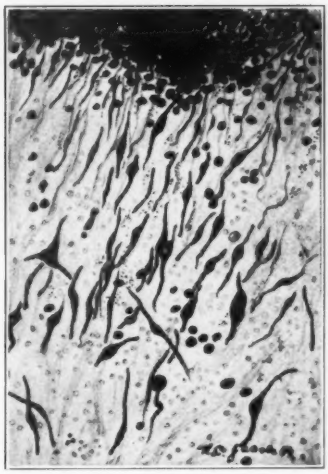
PLATE 77

FIG. 5. Small pigmented flecks in the pia. The chromatophores are seen radiating out from the center. Snessarew's modification of Cajal's technique. × 75.

FIG. 6. Drawing to show the various pigmented cells which form the pigmented fleck. Van Gieson's stain. × 75.

FIG. 7. Drawing to illustrate the development of the spindle-shaped chromatophore. 1 = round pigmented cell; 2 = oval pigmented cell without processes; 3 = oval pigmented cell with short processes; 4 = young spindle-shaped chromatophore; 5 = mature chromatophore; 6 = giant chromatophore with double nuclei. Unstained. × 300.

5



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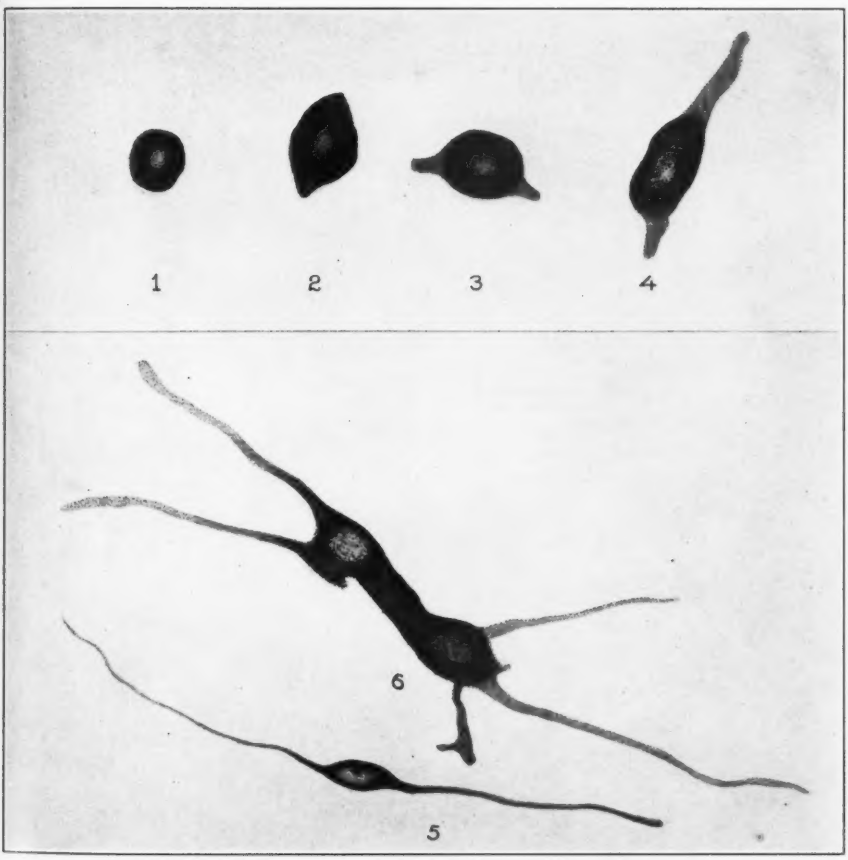

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Plate 78

Fig. 8. Drawing illustrating the development of the stellate chromatophore. 1 = round cell; 2 = polyhedral cell without processes; 3 = polyhedral cell with short processes; 4 = the mature chromatophore; 5 = chromatophore with double nuclei. Unstained. × 430.

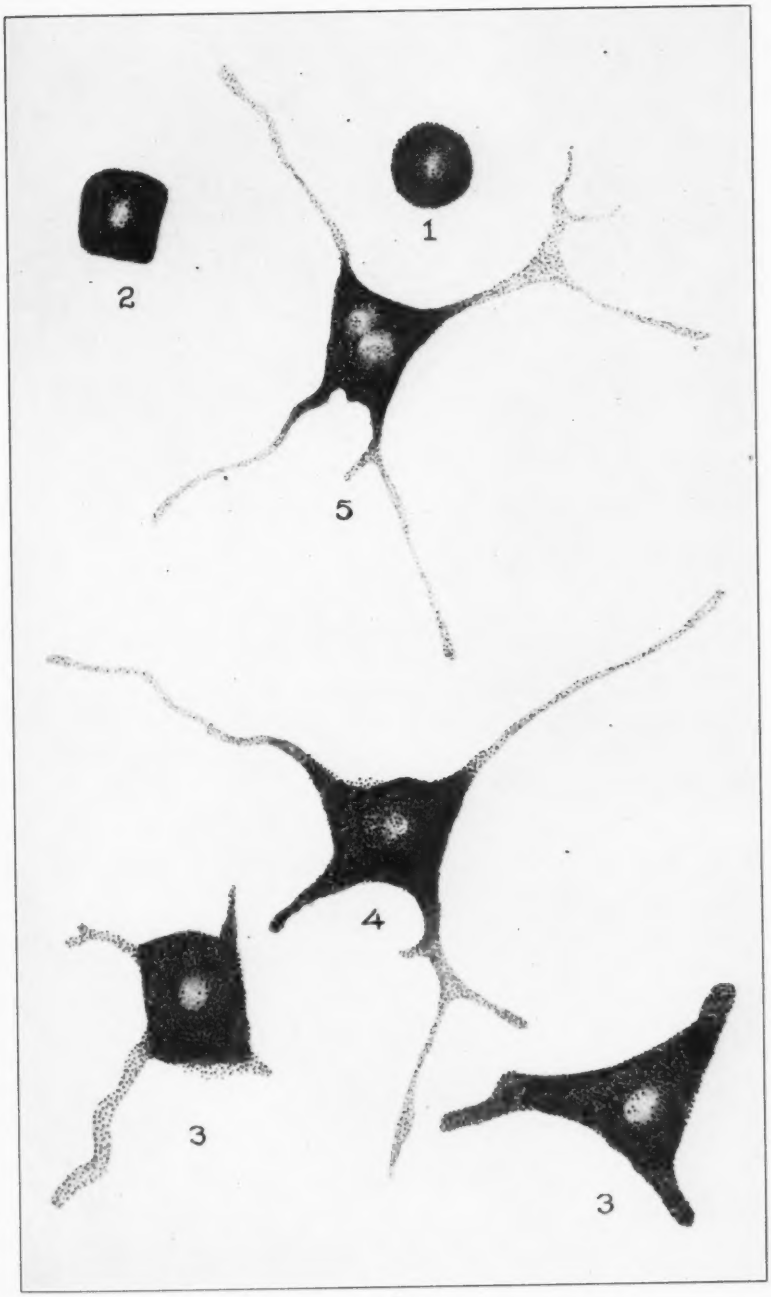

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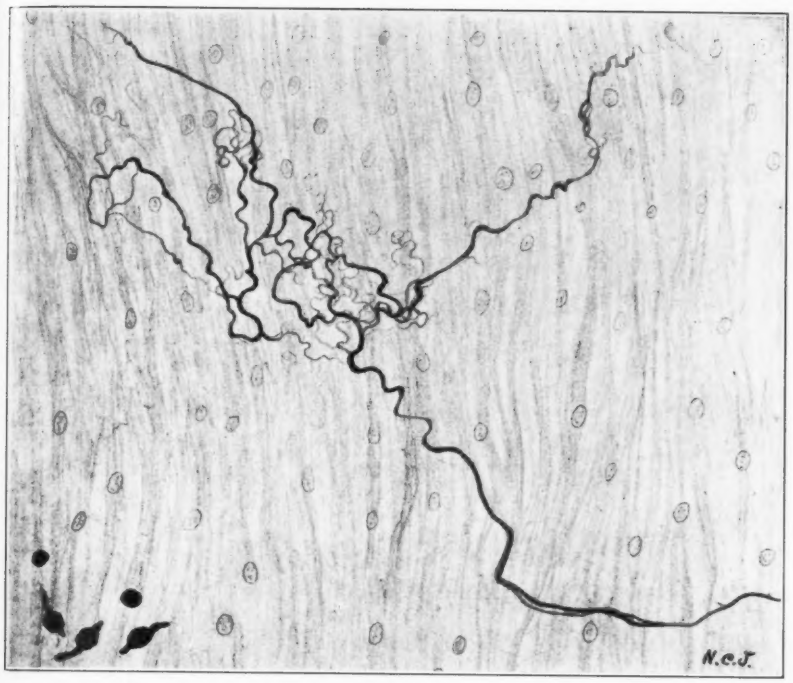
PLATE 79

FIG. 9. Drawing showing a nerve ending in the pia. Snessarew's modification of Cajal's technique. × 200.

FIG. 10. Tumor nodule in the brain parenchyma. The infiltrative nature of the tumor into the surrounding brain substance is well shown at the periphery of the tumor. This infiltration occurs through the perivascular spaces and is limited to the cortex. The meninges show moderate infiltration. The dilated blood vessels are outlined by collars of pigmented cells. Hematoxylin-eosin stain. × 20.



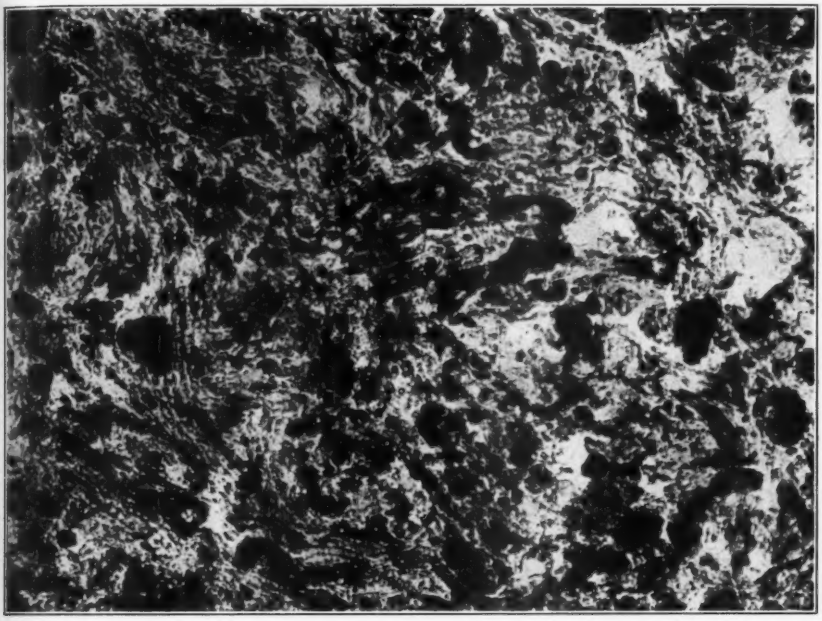
9

10

PLATE 80

FIG. 11. Field in the periphery of the tumor nodule showing the whorl-like formation of the tumor composed of spindle-shaped chromatophores. The perivascular spaces are filled with various types of pigmented tumor cells. Hematoxylin-eosin. × 100.

FIG. 12. Interior of tumor in the cortex. The sarcomatous appearance is due to the compact arrangement of the spindle-shaped chromatophores. Numerous round and polyhedral pigmented cells are scattered throughout. Van Gieson's stain. × 430.



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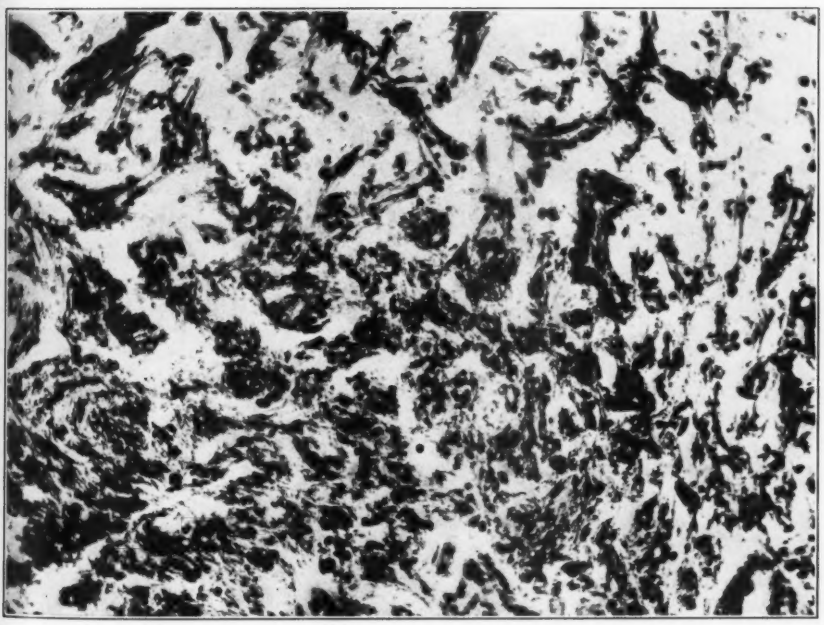
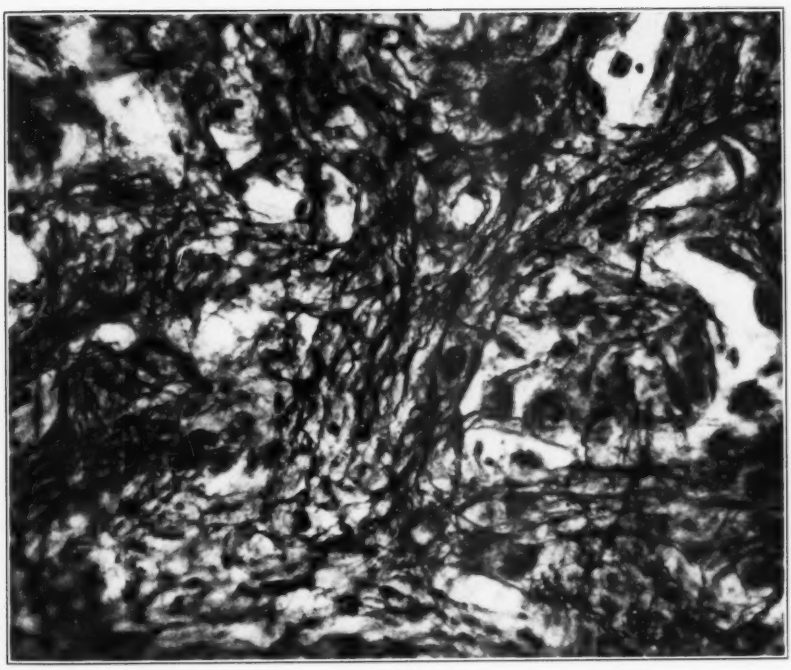


11

Akelaitis Primary Melanosarcoma of the Leptomeninges

Plate 81

Fig. 13. Field near the periphery of the tumor in the parenchyma showing reticulum and collagen fibers. The melanin granules are greatly decolorized. Perdrau's stain. × 430.



13

Akelaitis Primary Melanosarcoma of the Leptomeninges

THE INFLUENCE OF ANAPHYLACTIC SHOCK ON THE FINER STRUCTURE OF THE LIVER IN THE DOG *

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The symptoms of anaphylactic shock in the dog have been described so often as to become classical. Much is known, also, of the various physiological manifestations of anaphylaxis. But the histological descriptions are few, meager, and usually incidental to physiological or immunological studies. There is no adequate record of the finer histological or cytological details. It is my intention to describe some of the finer changes in the liver of the dog in anaphylactic shock and to attempt, at least, to correlate some of these changes with the already known physiological facts.

MATERIAL AND METHODS

Medium sized dogs (7 to 12 kg. body weight) were used for the experiments. Care was exercised in their selection, irritable and nervous animals being discarded.

White of egg, diluted 1: 10 with distilled water and filtered, was injected subcutaneously in 5 cc. doses on 3 successive days, and after a sensitization period of about 3 weeks the dogs were prepared for experimentation. Food was removed in the late afternoon to insure that the cellular pictures of the liver would be approximately the same in all dogs. The following morning a long glass cannula was inserted, under local anesthesia, into the thoracic duct. The rise of lymph in the cannula was measured for a period of an hour. Then 20 cc. of the diluted eggwhite was injected intravenously. Almost immediately the dogs began to show symptoms of uneasiness and quickly went into shock. Individual dogs act differently, but usually there is a period of primary shock followed by signs of improvement or recovery. Generally, however, the period of improvement is of short duration, and is followed by a secondary shock, more severe and lasting longer than the primary one.

* The experimental part of this study was done in the Pathological Laboratory of the University of Illinois, College of Medicine, Chicago. I desire to thank Professor William F. Petersen for the facilities which were afforded me.

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At different times, usually when the visible effects of shock had begun to cease, hepatic tissue was removed for microscopical study. An opening into the abdominal cavity was made under local anesthesia and in most cases a cannula was inserted into one of the large tributaries of the portal vein, instead of directly, so as to cause no interruption of the hepatic circulation. Sodium chloride in 0.9 per cent aqueous solution or a 10 per cent aqueous solution of sucrose at 38° C. was perfused through the liver and allowed to escape through an opening in the inferior vena cava above the diaphragm. Precaution was taken not to permit the pressure of the perfusing fluid to rise appreciably above the normal blood pressure of the dog. When the perfusing fluid began to escape only slightly tinged with blood the dog was killed with ether. The perfusion was continued for a short time, and then was followed by a warm mixture of 80 volumes of a 3 per cent aqueous solution of potassium dichromate and 20 volumes of formaldehyde solution. After some quantity of the fixation fluid had passed through the liver, the inferior vena cava and the portal vein were closed by ligatures. The liver was bathed *in situ* with the fixation solution, and in a half to 1 hour it was removed and small pieces cut out and put into fresh solution for 3 days. The pieces were then placed in an aqueous solution of 3 per cent potassium dichromate which was changed on alternate days for a week. In other dogs the livers were not perfused, but small pieces were cut out and fixed immediately. Material from the livers of normal dogs was treated in a manner similar to that from dogs in anaphylactic shock.

Serial sections were cut 5μ thick from material embedded by the celloidin-paraffin method. The stains employed were Heidenhain's iron-hematoxylin, Mallory's phosphotungstic acid hematoxylin and Volkonsky's modification of the Altmann anilin - acid fuchsin method. Satisfactory and comparable results were obtained with all three stains. In addition some sections were stained with hematoxylin and eosin and by the Heidenhain "azan" method.

Observations

The Liver in Primary Anaphylactic Shock: The injection of antigen into a sensitized dog is followed quickly by an acute congestion of the liver and a marked fall in arterial blood pressure. The histological picture is one of passive hyperemia. In livers fixed within

15 minutes after injection, the sinusoids and central veins of the hepatic lobules are dilated widely. The sublobular and larger tributaries of the hepatic veins are found wide and patent. Less dilatation is observed in the branches of the portal vein. Beneath the capsule of Glisson and in the loose connective tissue of the portal spaces, also around the central veins, there occur small patches of hemorrhage.

Generally the endothelial lining of the hepatic sinusoids shows no morphological alterations. In places, however, the endothelium is thrust away from the parenchyma, and fine reticular fibrils bridge the space. Some polymorphonuclear neutrophile leukocytes and a few free histiocytes (reticulo-endothelial cells) are present in the sinusoids, besides in and around the central veins. A few erythrocytes may be seen between the sinusoidal endothelium and the hepatic parenchyma.

Concomitant with the passive hyperemia of the liver there occurs a marked increase of lymph-flow from the cannula inserted into the thoracic duct. Direct measurements of the lymph-flow reveal as much as an 8.5 times increase in the hour following the injection of antigen over the hour preceding. This lymphagogue effect begins immediately upon injection and reaches a maximum in 7 to 15 minutes, then diminishes, but the pre-injection level is not reached before 1 or more hours. There is an accumulation of lymph between the sinusoidal endothelium and the hepatic parenchyma: the lymphatic vessels in the walls of the central veins and larger tributaries of the hepatic veins are dilated widely, and considerable dilatation of the lymphatic vessels in the portal spaces occurs.

These early stages of primary anaphylactic shock display little parenchymatous damage. The hepatic cells in the central zone of the lobules reveal some swelling, their cytoplasm shows more granulations, and there is the beginning of "cloudy swelling." Cells adjacent to the central vein often show vacuolization of the cytoplasm. Karyorrhexis, or even loss of nucleus, was observed in a few instances.

Parenchymatous changes are not of the same degree in all livers. Individual dogs react differently, but the sequence of hepatic alterations is very similar in all animals.

The Liver in Secondary Anaphylactic Shock: Generally after 10 or 15 minutes, although the time may be prolonged a little, the dog in

shock begins to present a second series of symptoms. These symptoms are usually more severe than those of primary shock.

Marked swelling of the parenchyma is a feature of this stage. Considerable differences, however, are noted in individual hepatic cells. A large number of cells in the central zone of the lobule exhibit varying degrees of swelling, some being greatly swollen, while others show swelling to a lesser degree or not at all. In severe shock the swelling may extend into the midzone, but not even in dogs dying in shock was any disorganization observed in the peripheral zone of the lobule.

The swollen parenchymatous cells undergo typical "cloudy swelling": the cytoplasm shows more apparent granulation. The granules through imbibition become enlarged into minute vesicles, the centers exhibiting diminished reaction to acid dyes, while the peripheries stain well. A later coalescence of vesicles leads to vacuole formation. Most cells in the central zone of the lobule, and a large number of those especially in the first part of the midzone, appear vacuolated. In certain cells the vacuoles reach some size, leaving but a small area of cytoplasm around the nucleus and thin septa between the vacuoles. As a sequel to "cloudy swelling" hydropic necrosis sets in. Large numbers of cells in the central zone and inner parts of the midzone display vesicles containing the hyaline balls from hydrops, which stain well with eosin and Mallory's phosphotungstic acid hematoxylin.

Accompanying these cytoplasmic changes, the nuclei of some of the parenchymatous cells undergo progressive alterations. The changes are confined mainly to a few rows of cells surrounding the central veins. First the nuclei stain less well, then they become shattered, and finally there is a complete dissolution of the nuclear remnants.

The extensive swelling of the hepatic parenchyma leads to a narrowing of the sinusoids. Practically all of the sinusoids in the central zone of the lobule are clamped down, and there is a diminution in caliber usually of those in the midzone. The central veins and the tributaries of the hepatic veins, likewise, are diminished in caliber. Near the periphery of the lobule the sinusoids are considerably wider. In the narrowed sinusoids, some of which are so reduced that erythrocytes can pass through only in single file, there is a stasis of blood. Later the individual erythrocytes lose their identity. The

central sinusoids thereby are plugged by hyaline masses. Often the same is true of the much reduced central veins.

The endothelial lining of the sinusoids is pushed away from the hepatic cords, so that the Kupffer cells and endothelial cells stand out prominently. The reticular fibers of the Kupffer cells are clearly discernible. Sometimes erythrocytes and polymorphonuclear neutrophile leukocytes lie between the endothelium and the parenchyma. Occasionally the endothelial lining is broken. In places the endothelium seems thickened; whether the thickening is real or merely a deceptive appearance cannot be said. Near the periphery of the lobule, where the sinusoids are considerably wider, the endothelium adheres more intimately to the hepatic cells.

Within the sinusoids, also in the loose engorged connective tissue around the central veins and tributaries of the hepatic veins, are found numerous polymorphonuclear neutrophile leukocytes, a considerable number of free histiocytes, and a few lymphocytes. Cells of all these types are more numerous than during primary shock. The Kupffer cells and the histiocytes display considerable vacuolization. Some of the vacuoles contain a yellowish brown pigment or whole erythrocytes and those in various stages of disintegration.

Many of the hepatic cells in the centers of the lobules have taken up whole erythrocytes. An individual hepatic cell may contain from one to (by actual count) thirty erythrocytes within its body. This taking up of erythrocytes leads to much enlargement of the cells, the nuclei being pushed eccentrically. The number of hepatic cells taking up erythrocytes is proportional to the severity of the shock.

Preparations subjected to the Prussian blue reaction reveal a considerable amount of iron in some Kupffer cells, histiocytes and hepatic cells. The liver of the normal dog gave but a slight reaction. This increased reaction for iron becomes significant when the ingestion of erythrocytes and their subsequent disintegration are taken into account. But it must be borne in mind that the increased reaction for iron does not mean necessarily that all the iron has been derived from erythrocytes. Anaphylactic shock may have unmasked iron which is normally present and which is not displayed ordinarily.

An interesting feature of the dog's liver is the remarkable development of smooth musculature around the tributaries of the hepatic veins. The sublobular veins are surrounded more or less completely

by a thick layer of muscle, while the larger hepatic tributaries display more muscle tissue on one side than the other.

Chondriosomal Changes: Since chondriosomes are the easiest and earliest altered of the cytoplasmic inclusions, changes in these organoids may be expected in anaphylactic shock. Morphological descriptions of chondriosomes are beset with difficulties because of differences in functional conditions and in methods of technique. Nevertheless, certain changes in chondriosomes may be seen under both physiological and pathological conditions when the tissues are compared with those of normal animals kept under identical circumstances.

The hepatic lobule of the dog may be clearly marked out by the morphology of chondriosomes into three characteristic zones, although some differences are shown within each zone. In the liver of the normal dog chondriosomes are present in the parenchymatous cells in the form of spherules, rods and filaments. Suitably fixed and stained preparations show an abundance and fairly even distribution of chondriosomes. In dogs without food for 18 to 20 hours the hepatic cells in the central zone of the lobule contain a predominance of spherules, variable in size, although short rods are often present, while in individual cells filaments may be seen lying between the spherules and rods. Generally the cells, three or four deep, surrounding the central vein contain almost exclusively spherical chondriosomes or mitochondria. The chondriosomes in the midzone of the lobule are for the most part longer than in the central zone. Filaments abound, but some rods of different lengths and spherules are to be seen in many cells. In the peripheral zone of the lobule filaments — usually longer and thinner — appear in abundance. Individual cells, however, may contain a large number of rods and spherules. This is especially true of the most peripheral layer of hepatic cells adjacent to the portal spaces, where often are present spherules of greater diameter than found elsewhere in the lobule. Also, certain lobules have been observed in which cells abutting the portal spaces contain exclusively filamentous chondriosomes. The lability of these organoids makes it difficult to state just what is a normal appearance.

During the different stages of anaphylactic shock, and corresponding with the various degrees of shock, many alterations of chondriosomes occur in the hepatic cells. In mild or in primary

shock many of the spherical chondriosomes in the central zone of the lobule become swollen and vacuolated. Individual chondriosomes display clear centers and peripheral stained hulls. Sometimes the stained part is all on one side like a crescent. Diminution of staining capacity is very evident, and in some of the most central cells there is a complete loss of mitochondria by chondriolysis. In the midzone and peripheral zone of the lobule there is little or no change in the chondriosomes, except in certain cells of the midzone where they appear as swollen spherules and rods.

Severe anaphylactic shock causes greater alteration of the chondriosomes. In the hepatic cells surrounding the central vein of the lobules there may be a complete loss of mitochondria, depending upon the extent of the central necrosis. Further out, accompanying the "cloudy swelling," the changes in the chondriosomes go hand in hand with the general swelling of the granules of the ground cytoplasm and the vacuolization of the cells. Rods increase in caliber and segment into spherules; these swell, vacuolate, and some undergo dissolution. Cells filled with numerous or large vacuoles show a dispersion of chondriosomes between the vacuoles, around the nuclear membrane, and toward the periphery of the cells. In the midzone, particularly in the inner part, the filamentous chondriosomes are segmented mostly into rods and spherules. As a rule the degeneration of chondriosomes does not extend very far peripherally. Chondriosomes in the outer part of the midzone and in the peripheral zone, except in certain "dark cells," appear quite similar to those in the hepatic cells of normal dogs.

It is interesting to follow in some detail the sequence of degeneration of chondriosomes. Filaments (chondriocontes) may become beaded along their whole length, or in the middle, or may present blebs at one or both ends. Segmentation may occur between the beads, or the beads may swell and burst, thus shortening the filaments. A similar process occurs in the rods. Spherules (mitochondria) degenerate, first by swelling and vacuolization, followed by shattering and dissolution of the fragments. Chondriolysis, as here described, is usually an accompaniment to karyorrhexis and karyolysis. Another form of degeneration of chondriosomes will be recounted in connection with certain "dark cells."

Dark Cells: The literature abounds with descriptions of "dark" and "light" cells in the liver. In the liver of the normal dog both

dark and light cells are found in varying numbers. At the periphery of the hepatic lobule a single row, occasionally in places two cells deep, of dark cells lies tangential to the portal spaces and sometimes extends out along the interlobular vessels. In addition single dark cells or small groups of two, three, or four cells may be present almost anywhere in the lobule, especially in the midzone and peripheral zone. Anaphylactic shock appears to cause an increase of dark cells in both of these situations.

The peripheral or tangential dark cells are to be distinguished from the hepatic cells further in the lobule by their tinctorial reactions and by the character of their non-vacuolated, more or less homogeneous cytoplasm. In ordinary hematoxylin and eosin preparations the cytoplasm displays an intense affinity for the acid dye, besides often showing a distinct basophilic tinge. Mallory's phosphotungstic acid hematoxylin and Heidenhain's iron hematoxylin reveal an abundance of chondriosomes in the peripheral dark cells, so they may be spoken of as *chondriosome-rich* cells. From their location in the lobule, from their size and shape, and from nuclear and cytoplasmic characteristics these cells are without doubt hepatic cells. No transitional forms have been observed between them and the epithelial cells lining the intrahepatic bile ducts. Since the branches of the hepatic artery enter at the periphery of the lobule, it is reasonable to assume that these peripheral or tangential dark cells are better nourished than the cells further in. This increased nourishment may account, in part, for the abundance of chondriosomes. In many preparations the chondriosomes are present in the form of long slender filaments, but frequently the filaments are replaced by spherules, approximately twice the diameter of those in the hepatic cells around the central vein. The possible significance of these large spherules will be taken up later in the discussion. There can be scarcely any doubt that the peripheral dark cells are normal, because they display neither cytoplasmic nor nuclear signs of degeneration. Mitoses have been encountered in the peripheral dark cells, also cells have been observed with two nuclei.

The other more scattered form of dark cells, on the contrary, shows signs of both cytoplasmic and nuclear degeneration. The extensive destruction of the hepatic parenchyma in the center of the lobule incident to anaphylactic shock leads to a proliferation of cells further out, as attested both by mitotic figures and by the increment

of binucleate cells. Other cells, especially in the midzone and peripheral zone of the hepatic lobule, being perhaps toward the end of their cytomorphosis, undergo degeneration. These last cells display an enlargement of chondriosomes, which later become clumped together so that individual forms are not discerned, or are seen with difficulty. Accompanying the changes in cytoplasmic inclusions the nuclei undergo pyknosis. The cells become shrunken, irregular, and both nuclei and cytoplasm stain uniformly dark. Finally, only dark staining masses lie amid cells of perfectly normal appearance. These degeneration cells have been observed also on occasion lying between the undegenerated peripheral dark cells.

Discussion

It is well established that anaphylactic shock in the dog is associated with marked circulatory disturbances which presumably are located on the venous side. The passive congestion of the intestines and liver, whether because of a relaxation of the capillaries which increases the capacity of these organs, or from some impediment to the outflow from the liver, causes an insufficient return of blood to the heart. Coincident with this passive congestion there is a marked fall in arterial blood pressure.

Manwaring [1] pointed out the importance of the liver in the production of anaphylactic shock in the dog. Voegtlin and Bernheim [2] and Denecke [3] supported Manwaring's view, because when the liver was isolated from the circulation by an Eck fistula and ligature of the hepatic artery, it was impossible to produce the anaphylactic state. Subsequent investigators, while recognizing the rôle of the liver, have not been in agreement as to the mechanism involved by which this organ participates.

It was thought by Manwaring [1] that vasodilator substances liberated by the hepatic parenchyma act upon the systemic blood vessels and bring about a reduction of blood pressure. Biedl and Kraus [4] believed that toxic peptone-like bodies derived by proteolysis from the injected antigen into a sensitized dog caused the fall in blood pressure, through a paralysis of the vasoconstrictor nerve endings. Weil [5] was against the idea of a circulating toxin and assumed that reactions taking place in the sensitized hepatic cells are responsible for anaphylactic shock in the dog. The congestion of the liver was considered secondary to irritation of the parenchyma.

On the contrary, Simonds [6] attributed the passive congestion of the liver in anaphylactic and peptone shock in the dog to a local venous constriction, caused by a spasm of the extensively developed smooth musculature in the walls of the hepatic veins and their tributaries. Mautner and Pick [7] had shown that the hepatic veins of dogs were constricted by histamine. The existence of these atypical veins in the liver of the dog is well established and their spasmodic closure would help explain some of the similarities of anaphylactic, peptone and histamine shocks. But several investigators have entered objections to the theory of Simonds. Manwaring and Brill [8] were unable to demonstrate a veno-constriction when a mixture of epinephrin and barium chloride, ergotin, or Vaughan's protein split-product was perfused through the isolated liver of the dog. Manwaring and his associates [9] considered that one of the important factors in anaphylactic shock in the dog was increased permeability of the sinusoidal endothelium. An explosive edema accompanied by swelling of the parenchymatous cells of the liver increases local tissue pressure sufficiently to cause passive constriction of the sinusoids and hepatic veins. A stasis of blood in the sinusoids and narrowed hepatic veins is brought about by the increase of viscosity through the loss of fluid. Leukocytic deposits may be a minor factor in increasing the resistance.

Petersen and his collaborators [10, 11, 12] have taken the similar stand, that anaphylactic shock in the dog consists primarily of an endothelial shock. As the result of stimulation, or of irritation, the permeability of the endothelium is increased, especially in the splanchnic area. An enormous quantity of fluid is forced into the hepatic lymphatics, distending the liver, disorganizing the capillaries, and allowing the escape of blood corpuscles into the lymph. The specific protein to which the cells have been sensitized leaves the capillaries rapidly and comes in contact with the parenchymatous cells, resulting in a primary shock. The passive congestion produces more injury, giving rise to a secondary shock. An "endothelial blockade" accomplished by injections of saccharated oxide of iron alters the intensity of the shock, either as a true blockade, or by increasing the activity of the endothelium, involving perhaps a more rapid destruction of the antigen, thereby protecting the parenchymatous cells.

Finally, Zinsser [13] in summary wrote: "While we have no positive

knowledge of the site of the anaphylactic reaction within the body, it is more than likely that the primary point of attack is in the reticulo-endothelial system." Even if this supposition be true, we do not know whether the antigen injected into a sensitized dog acts directly upon the endothelium, increasing its permeability, or upon nerve endings, affecting the vasomotor mechanism. A study of the possible influence of the nervous system in the production of anaphylactic shock is now in progress.

How is the remarkable increase of lymph-flow from the thoracic duct during anaphylactic shock to be accounted for? The well known conclusions of Starling [14] that "the whole increase of lymph obtained on obstruction of the inferior vena cava above the diaphragm is derived from the liver," have not been substantiated fully by more recent investigators. Markowitz and Mann [15] thought that the liver, while contributing a part of the lymph to the thoracic duct, played a lesser rôle than supposed by Starling. When the periportal lymphatics were ligated no change was noticed, except a temporary increase of lymph-flow, which could be attributed to the intestines and operative manipulations. After the removal of the liver, there was found no diminution of lymph-flow. Peptone produced the usual lymphagogue effect in the hepatectomized dog, in which Markowitz and Mann considered that the lymph came largely from the intestines. Drinker and Field [16] are inclined, likewise, toward the idea that the liver is not, but the intestines are, the main source of lymph from the abdomen.

The increase of intraportal pressure, owing to the congestion of the liver and other organs of the splanchnic area, favors the passage of fluid out of the capillaries as lymph. Asphyxia is an early symptom of acute anaphylactic shock; this deprivation of oxygen to the tissues would increase capillary permeability and facilitate the flow of lymph. While we can argue only from analogy, the chemical studies of Petersen and his associates are convincing that anaphylactic shock in the dog alters the lymph picture and strongly suggests an increase of capillary permeability.

Serial sections of the liver of the dog reveal two sets of lymphatic vessels, — one in the portal spaces, the other around the tributaries of the hepatic veins, and none within the hepatic lobules. Similar observations have been pointed out by Herring and Simpson [17] in the dog and cat, and by Lee [18] who ligated the thoracic duct in the

cat and obtained a retrograde injection of the lymphatics in the liver. Since the liver has a dual drainage, part to the hepatic lymph glands and part to the diaphragmatic lymph glands, it seems reasonable that some lymph from the organ never reaches the thoracic duct, but flows eventually into the right lymphatic duct. For this reason, and because of the congestion of the intestines, in anaphylactic shock we have no idea how much of the increase of lymph-flow from the thoracic duct is derived from the liver.

The congestion of the sinusoids and efferent veins of the liver and the swelling of the parenchymatous cells in the centers of the hepatic lobules in primary anaphylactic shock confirm the observations of Weil,[5] Manwaring, French and Brill,[9] and Dean and Webb.[19] It is only in secondary shock, where an increased swelling and necrosis of the cells is accompanied by perivascular edema, that a narrowing of the sinusoids, hepatic veins and their tributaries occurs. Mallory,[20] in introducing the term central necrosis, described a comparable picture of the liver, and thought that this type of necrosis was brought about by toxic action. Since the parenchymatous cells in the centers of the lobules are not so well nourished as those toward the periphery, being further removed from the blood brought in by the hepatic artery, they are more labile and susceptible to injury. Later observers, as Zimmerman and Hillsman,[21] Bolton and Barnard,[22] and Simonds and Callaway [23] agree with Mallory that the centers of the hepatic lobules are less resistant because they are removed further from the source of nutriment, but doubt that toxic action plays a rôle in the production of central necrosis from venous stagnation.

From the above accounts it would appear that uncomplicated venous stasis in the liver causes injuries to the parenchymatous cells, which parallel those brought about by anaphylactic shock. There occur the same parenchymatous swelling, karyorrhexis and karyolysis, followed by cytolysis of the cells in the central zone. The sinusoids are narrowed by the swelling of the parenchyma and the perivascular edema, and hyaline thrombi appear in many places. But the presence of focal necroses in the midzone and peripheral zone of the hepatic lobule, in which the cells undergo homogeneous atrophy, does not occur in cases of simple passive congestion of the liver. Such necroses were looked upon by Flexner,[24] Mallory,[20] Pearce,[25] Fiessinger, [26, 27] and Karsner and Aub [28] as toxic in origin.

I am of the same opinion, and in addition regard the extensive lesions in the centers of the hepatic lobules in secondary shock as of toxic origin. This view is shared by Weil, Manwaring, Dean and Webb, and others from observations on anaphylactic shock in the dog, and by Apitz [29] in the rabbit. Furthermore, how are we to explain the clamping down of the hepatic veins and their tributaries on the ground of simple uncomplicated passive congestion? Here again it seems that there must be some toxic product liberated in anaphylactic shock which acts upon the extensively developed smooth musculature in these veins, since constriction was observed only in secondary shock. Finally, the taking up of whole erythrocytes by the hepatic cells, a feature of severe anaphylactic shock in the dog, is unknown as a sequel to passive hyperemia of the liver. Browicz [30, 31] in a series of papers reported the presence of whole erythrocytes in the hepatic cells of the dog following intravenous injections of hemoglobin and of defibrinated blood. He accepted the existence of intracellular canaliculi communicating with the sinusoids, a view that has not remained unchallenged. Rössle,[32] in a case of human hepatic cirrhosis, observed erythrocytes not only in the parenchymatous cells of the liver, but also in the pancreas and kidney. Capillary failure was attributed as the cause. The evidences of increase of capillary permeability, disorganization of the sinusoidal endothelium and parenchymatous necroses detailed in severe anaphylactic shock in the dog make it quite possible that erythrocytes may pass either by diapedesis through the endothelium or directly into the injured hepatic cells. The increment of iron seen by me, and described by others, in the Kupffer cells and parenchymatous cells is at least suggestive in this connection.

Mayer, Rathery, and Schaeffer [33] studied the degeneration of chondriosomes in the liver and emphasized the ease with which these cytoplasmic inclusions are altered. They pointed out two types of alterations, namely, by cytolysis and by homogeneous atrophy. Both of these types of chondriolysis were stated to occur after various poisons, mineral and organic, also as the result of microbic toxins and those of autolysis. Similar types of degeneration present themselves in the hepatic cells of the anaphylactic dog. In the first type the chondriosomes, particularly in the central zones of the hepatic lobules, undergo segmentation, round off and become transformed into vesicles with clear centers. A complete dissolution is

the final outcome. This type of chondriolysis manifests itself in the cells where "cloudy swelling" is most evident. Smith and Rettie [34] reported similar observations. It is probable that some of the hypertrophied granules mentioned by Landsteiner [35] in his study of "cloudy swelling" are likewise mitochondria. Hypotonic solutions according to Bang and Sjövall,[36] Anitschkow,[37] Smith and Rettie,[34] and others produce the same type of chondriosomal degeneration. It would appear that the imbibition of fluid from the ground cytoplasm causes the chondriosomes to swell, lose their staining capacity, and finally to undergo dissolution.

The homogeneous atrophy of chondriosomes was observed only in dark cells of the degeneration type. These cells, confined mostly to the midzone and peripheral zone of the hepatic lobule, are without doubt toward the end of their cytomorphosis. Fiessinger [27] included them in his general group of dark cells, for which he offered three interpretations: (1) artifact of preparation; (2) state of functioning; (3) pathological alteration. That the cells are artifacts he thought could be dismissed, because with the same fixation hepatic dark cells are found rarely in the guinea pig and frequently in the dog. Moreover, using mitochondrial methods, chondriosomes are brought out very clearly and evenly spaced in the light cells, whereas they are condensed and lie close together in the dark cells. Cohn [38] distinguished light and dark cells in the liver of the mouse, dog, cat, rabbit and guinea pig, and thought that the two appearances represented a different functional state. A number of authors from inanition studies have concluded the same thing, because in a state of complete inanition the number of dark cells is increased, while in well nourished animals their number is reduced or absent. I believe that this is true, as regards the peripheral dark cells, but the condition of the chondriosomes in the more scattered form is evidence to the contrary. The third interpretation, that the dark cells represent pathological alterations, has the support of the experimental studies of Fiessinger,[26] Policard,[39] Fiessinger and Lyon-Caen,[40] and several later workers. From the appearances that I have observed in the livers of dogs in different stages of anaphylactic shock, and shock of varying degrees of severity, I think that there are two types of dark cells; one type, clearly degenerative, may be derived either from light cells or from the other type, the peripheral or tangential dark cells. These peripheral or tangential dark cells, distinguished by

Rumjanzev,[41] Kutsuna,[42] Rabl,[43] Böhm,[44] Clara,[45] Pfuhl,[46] and others represent without much doubt normal cells. The chondriosomes in these dark cells, besides being very abundant, appear normal. The suggestion of Böhm[44] and of Pfuhl[46] that these cells represent functional states is timely, because the periphery of the hepatic lobule is better nourished, receiving blood from both branches of the hepatic artery and portal vein.

In certain cells or groups of cells on or near the periphery of the hepatic lobule, particularly adjacent to the portal spaces, large spherical chondriosomes occur having about twice the diameter of the spherules in the cells surrounding the central vein. Because of the location of these cells it was considered that a stasis of bile might account for the appearances of the chondriosomes. Fiessinger and Lyon-Caen[47] observed the transformation of rod-like chondriosomes into spherules when a hypersecretion of bile was provoked in the dog by intravenous injections of hemoglobin. Bang and Sjövall[36] described chondriosomes becoming spherical when pieces of frog's liver were treated with bile. Albot[48] noticed the same effect after ligation of the common bile duct in the rabbit.

Summary

1. Anaphylactic shock in the dog presents two characteristic stages: (*a*) a primary shock, usually of short duration; and (*b*) a secondary shock, more severe and prolonged.

2. An extreme congestion of the liver, a marked fall in arterial blood pressure, and an increase of flow of lymph from the thoracic duct are displayed as features of primary shock. Some swelling of the hepatic cells appears in the centers of the lobules.

3. Secondary shock produces more damage to the parenchyma of the liver, as "cloudy swelling," hydrops, vacuolization of the cytoplasm, and finally a central necrosis. Accompanying these degenerative changes there is found a diminution in caliber of the sinusoids of the hepatic lobules, also of the efferent veins of the liver. A stasis of blood in the narrowed sinusoids leads to formation of hyaline plugs or thrombi.

4. A disorganization of the sinusoidal endothelium, proliferation of the endothelial cells, and increased phagocytosis by the Kupffer cells are very evident. The taking up of whole erythrocytes by the

parenchymatous cells of the liver is noted in severe secondary shock. Both the hepatic cells and Kupffer cells reveal an increment of iron.

5. Chondriosomes reflect the extent of parenchymatous injury. In the centers of the lobules spherical chondriosomes abound. Later, in some of the most central cells, chondriolysis occurs accompanying karyorrhexis and karyolysis. Homogeneous atrophy preceded by chondriomegaly appears in certain cells or groups of cells in the midzone and peripheral zone of the hepatic lobule. Two forms of "dark cells" may be differentiated on the basis of nuclear and cytoplasmic appearances.

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DESCRIPTION OF PLATES

PLATE 82

Figures 1 to 6 were drawn with the aid of a camera lucida at an approximate magnification of 360 × from preparations fixed in Regaud's potassium dichromate and formaldehyde mixture and stained by Mallory's phosphotungstic acid hematoxylin method.

FIG. 1. Normal dog. A sinusoid is shown entering into a central vein of the liver. Chondriosomes are pictured largely as spherules, while some rods and short filaments are present.

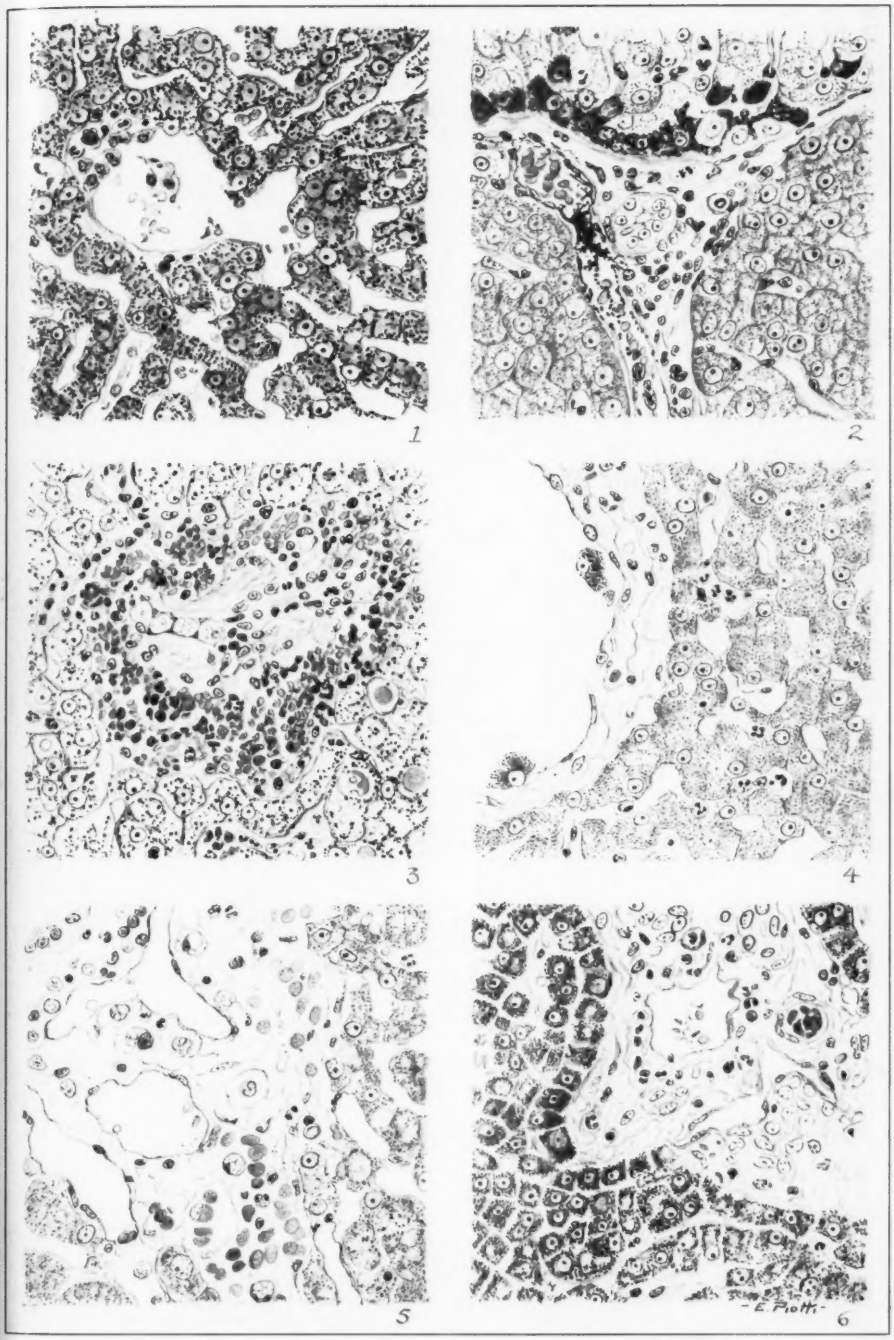
FIG. 2. Normal dog. Portal space. Tangential dark cells are shown at the periphery of a hepatic lobule.

FIG. 3. Anaphylactic dog. Secondary shock. A central vein is depicted filled with a hyaline mass and blood cells. Note the group of detached hepatic cells which lie within the lumen of the vein. Some hepatic cells appear swollen and contain hyaline balls of hydropic necrosis. The sinusoids show narrowing.

FIG. 4. Anaphylactic dog. Primary shock. The sinusoids are not narrowed. Pericentral lymphatics are distended. Free hepatic cells lie in the lumen of a central vein.

FIG. 5. Anaphylactic dog. Primary shock. Notice that the pericentral lymphatics display greater distention than those in Fig. 4. Erythrocytes may be observed within the lymphatic vessels. Histiocytes, leukocytes and erythrocytes are present in the connective tissue sheath of the vein.

FIG. 6. Anaphylactic dog. Secondary shock. Portal space. The lymphatic vessels show some dilatation. Tangential dark cells are portrayed mostly with filamentous and rod-like chondriosomes.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E. Plotti
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PLATE 83

Figures 7, 9, and 11 were drawn at an approximate magnification of 940 × and Figures 8, 10, and 12 of 1150 × from preparations fixed in Regaud's potassium dichromate and formaldehyde mixture and stained by Mallory's phosphotungstic acid hematoxylin method.

FIG. 7. Anaphylactic dog. Secondary shock. Hepatic cells adjacent to the central vein show spherical and rod-like chondriosomes, with beading of some of the longer forms.

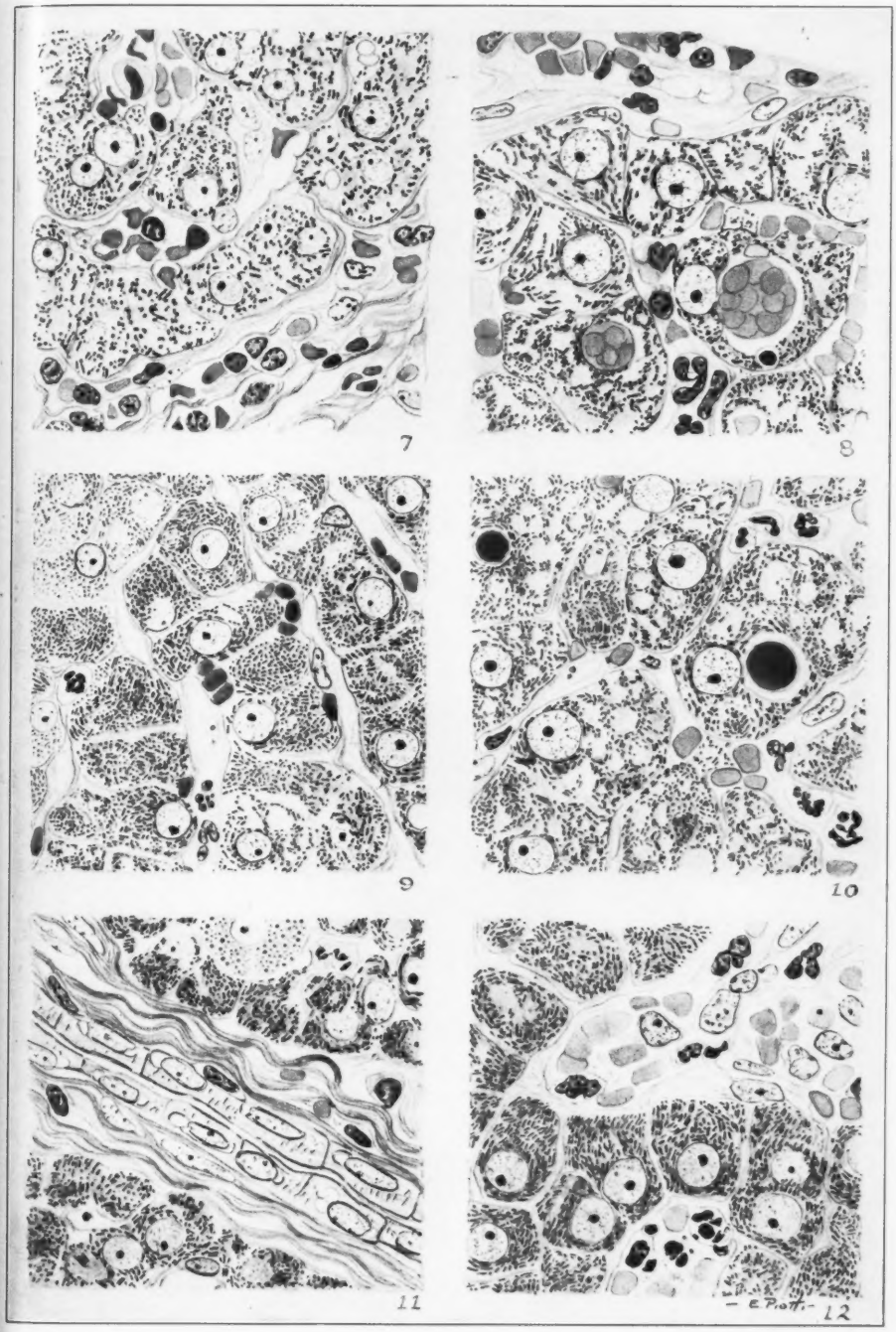
FIG. 8. Anaphylactic dog. Secondary shock. Central zone. Two hepatic cells display a taking up of erythrocytes.

FIG. 9. Anaphylactic dog. Secondary shock. Peripheral zone. "Dark" and "light" cells. Notice that there is no evidence of chondriolysis.

FIG. 10. Anaphylactic dog. Secondary shock. Midzone. Two cells show hydrops. Leukocytes abound in the sinusoids.

FIG. 11. Anaphylactic dog. Secondary shock. Section at the periphery of two lobules showing an intralobular bile duct cut longitudinally. Notice the abundance of chondriosomes present in the tangential dark cells.

FIG. 12. Anaphylactic dog. Secondary shock. Periphery of a lobule. The chondriosomes in the dark cells display a more or less proximodistal arrangement.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LESIONS IN THE ROOTS OF THE PULMONARY ARTERY AND AORTA IN RHEUMATIC FEVER *

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When blocks are cut from the heart according to the standardized technique suggested by Gross, Antopol and Sacks,[1] it becomes possible to study approximately 1.5 to 2 cm. of the aortic and pulmonic roots, *i.e.* the last musculo-elastic portions of the vessels prior to their transformation into connective tissue annuli. In the routine examination of a large number of active and inactive rheumatic hearts employing this standardized method, a surprisingly high incidence of pathological lesions was observed both in the roots of the pulmonary artery and aorta proper, as well as in the vessels within their enveloping pericardial mantles. The lesions occurring in the walls of the arch, and ascending and descending portions of the aorta † have been described in considerable detail by Klotz,[3] Pappenheimer and VonGlahn,[4, 5, 6] Chiari,[7] Shaw,[8] Giraldi,[9] Perla and Deutch,[10] McClenahan and Paul,[11] Gray and Aitken,[12] and Klinge.[13] Those occurring within the walls of the pulmonary artery seem, for the most part, to have escaped notice. The only reports available on this subject appear to be those by Paul,[14] who was able to substantiate Sacks' [15] prediction that such lesions would be found, and by Kugel and Epstein,[16] Shaw,[8] Gray and Aitken,[12] and Chiari.[17] None of these reports, however, lays special emphasis on the roots of these vessels. Kugel and Epstein appear to be the first to have called attention to the frequency with which lesions may be found in the "musculo-arterial junction" in the acute cases. By this term they referred to the region where the annulus is inserted into the myocardium, an area that properly belongs to the valve ring. They indicated the possibility that the dilatation of the aortic ring, which frequently occurs in acute cases, may bear some relation to the damage found early in this area.

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† Inflammatory nodules in the root of the aorta in rheumatic fever were observed by Coombs in 1908.[2]

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In this report it is proposed to describe the lesions found within the roots of the pulmonary artery and aorta proper, *i.e.* the purely musculo-elastic portions, as well as within the vessels found in their enveloping pericardial mantles. These lesions will be considered statistically with respect to their incidence in active and inactive rheumatic cases as well as in control material. A discussion will be given of their possible significance.

Material and Methods

The findings in 150 hearts form the basis of this report. Of these, 66 presented active rheumatic fever as manifested by the presence of fibrinous pericarditis, acute verrucous endocarditis, Aschoff bodies, eosinophilic collagen changes (fibrinoid) and other inflammatory phenomena in the myocardium. Thirty-four cases represented inactive rheumatic material, according to the criteria formulated by Rothschild, Kugel and Gross.[18] The remaining 50 cases were from a non-rheumatic control series representing various age periods from birth to the ninth decade of life. This series was carefully selected to eliminate past or present hypertension since this condition is associated with vascular changes in the periaortic and peripulmonic sheaths.* Syphilis was excluded by the history, corroborative pathological findings or positive Wassermann test. After fixation of the heart in 10 per cent neutral formalin saline,† blocks were cut according to the standardized method of Gross, Antopol and Sacks. Sections were cut from each block 7.5 μ thick and stained as a routine with hematoxylin and eosin and with Weigert's elastic and Van Gieson's connective tissue stains.

Root of the Pulmonary Artery Proper

In an examination of the non-rheumatic control hearts it was found that occasional capillaries were present between the medial fibroelastic and muscular strands of the pulmonary artery root in approximately 24 per cent of the cases. These capillaries are extremely inconspicuous and rarely penetrate inward beyond the outer third of the media. As compared to this, the root of the pulmonary artery

* The peripulmonic and periaortic sheaths mentioned in this report refer to the pericardial and adventitial mantles around the roots of these vessels.

† Solution of formaldehyde U. S. P., 10 parts; 1 per cent sodium chloride solution, 90 parts. This solution is rendered neutral with a weak alkali.

in the inactive rheumatic hearts presented capillaries in 70 per cent of the cases. These were not infrequently larger, somewhat irregular and penetrated at times into the middle third of the media, more rarely into the inner third (Fig. 1). There were no inflammatory cells seen surrounding these capillaries. At times, however, a scant amount of connective tissue was noted about them.

When this connective tissue around the capillaries becomes somewhat more conspicuous one may speak of "scarring" within the media. Medial scarring is usually associated with some change in the nature, amount and distribution of the elastic membranes. These may be absent, distorted, ruptured, frayed or even markedly increased. Moderate grades of scarring and alterations in the structure of the elastic membranes are difficult to discern in the root of the pulmonary artery because of the anatomical peculiarities of this site. Thus, in the normal pulmonary artery root there is a very considerable variation in the arrangement of the elastic lamellae, fibrous tissue and smooth muscle cells. In contrast to the histological structure of the aortic root, the region where the pulmonary artery proper passes into its annulus insertion often displays marked irregularities in the distribution and orientation of its elastic and muscular fibers which form an uneven mosaic with patches of fibrous connective tissue. The elastic fibers frequently take the form of spiral and circular arches. Because of these peculiarities in structure, cross-section of the pulmonary artery root is apt to present what appear to be discontinuities of the elastic membranes, and irregular areas of connective tissue devoid of smooth muscle or elastic fibers.

Another confusing element is the fact that the pericardial sheath surrounding the pulmonary artery root is generally in close apposition to it, especially during the early age periods. With increasing age, a looser fibro-adipose tissue makes its appearance. Within this pericardial mantle there are to be seen small arteries and arterioles. During the early age periods, and when the adipose tissue is relatively scant, the more fibrous and closely adherent pericardial sheath with its blood vessels is apt to present a picture that may be confused with early adventitial scarring of the pulmonic trunk and with mild invasion of its wall with blood vessels.

Bearing in mind the above mentioned histological peculiarities, it may be stated that in only two of the fifty control pulmonic roots examined did obvious scarring come into consideration. In 1 case,

careful study of the supposed lesion led to the conclusion that one was dealing with an exaggerated normal variant in the distribution of the elastic, smooth muscle and connective tissue fibers. The other case, however (a child 22 months of age with hilar and meningeal tuberculosis), had to be considered as showing scarring with considerable rupture of the elastic membranes. No inflammatory cells were present and the capillaries were scant. In the inactive rheumatic cases small flame-shaped scars were seen in three instances. These were, however, very inconspicuous and no inflammatory cells were present.

Apart from the question of capillaries and scarring in the pulmonic root, there was found no significant difference in the amount of chromotropic substance as between the control and the inactive rheumatic cases. Edema was not found in either of these groups nor were eosinophilic collagen changes present.

As is to be expected, the incidence and variety of lesions in the pulmonic root is considerably greater in the active rheumatic cases. Thus, as compared to capillarization in 24 per cent of control cases and in 70 per cent of inactive rheumatic cases, this lesion was noted in 80 per cent of the active cases. In one-sixth of these capillarized cases the capillaries were quite numerous and extensive in their distribution.

Three of the active cases showed moderate sized, irregular scars — 2 with marked elastica disruption (Fig. 2) and 1 with moderate elastica disruption. In another case the elastic fibers were tremendously increased in amount and localized in small, irregular patches.

Seven of the capillarized cases showed perivascular accumulations of generally large mononuclear or lymphocytic cells, at times, polymorphonuclear leukocytes. In 6 of these there existed a diffuse inflammatory infiltration of the pulmonic root of various grades. These consisted of an irregular distribution of, at times, large mononuclear cells with large round or oval nuclei containing dust-like chromatin granules and with deeply basophilic cytoplasm. The latter was often quite abundant and possessed irregular indistinct edges. These cells generally occurred in palisades between the elastic lamellae, such as described in the aorta by Pappenheimer and VonGlahn. In 1 case, the distribution of the cells was focal, somewhat resembling the structure of an Aschoff body. Together with these mononuclear cells, or at times in their absence, there also oc-

curred an irregular distribution of neutrophilic polymorphonuclear leukocytes, lymphocytes and occasional eosinophiles. Many of the polymorphonuclear leukocytes and monocytes showed an orientation in the direction of the vessel lumen.

As mentioned before, these cells were irregularly distributed, sometimes being accumulated in greater numbers toward the inner zone of the pulmonic root media, sometimes toward the middle or outer zone, sometimes quite diffusely. In each of these 6 cases of pulmonic arteritis there were present varying degrees of eosinophilic swelling of collagen fibers and often of the elastic lamellae. These were, at times, stretched by edema fluid and accumulated inflammatory cells. In places the elastic fibers seemed to have disappeared.

In these cases and in 3 additional ones the smooth muscle cells between the elastic lamellae appeared to be swollen, with the nuclei perhaps slightly more conspicuous. As a consequence, cross-section of these fibers gave the appearance of prominent rows of cells between the elastic lamellae.

In approximately one-third of the active cases there was present some form of intimal change. In 1 case there was noted a verrucous endarteritis (Fig. 3). This consisted of the formation of typical verrucous material within a localized proliferative intimal zone. The latter was situated internally to the innermost elastic lamellae and consisted of swollen, slightly and at times deeply basophilic stellate cells separated by mucin-like groundwork. This, in all probability, represents a proliferation of the subendothelial undifferentiated mesenchyme. The structure was covered by endothelial cells except for the central portion, which was the seat of the verrucous change.

In 2 other cases there was seen a similar intimal "reduplication" but without verrucous change. In these, however, there were present swelling and eosinophilic change of the collagen with accumulations of polymorphonuclear leukocytes and lymphocytes. In the remaining cases the intimal lesion consisted of a more mature intimal reduplication, *i.e.* one in which the stellate cells have largely transformed themselves into mature fibroblasts, collagen has been laid down and some elastification has occurred. In these stages these reduplications cannot be distinguished from similar changes due to age period processes.

Summarizing these findings in the pulmonic root proper it appears that rheumatic fever produces a considerable increase in the inci-

dence and extent of capillaries, and a definite though considerably smaller increase in the incidence of scarring and genuine elastica disruption; that inactive cases show no accumulation of inflammatory cells or edema and no eosinophilic collagen changes; that in about 14 per cent of the active cases all or some of the following manifestations of acute damage are present, *viz.* accumulation of large basophilic mononuclear cells, polymorphonuclear leukocytes, formation of palisades, edema, rupture or disappearance of elastic membranes, eosinophilic swelling of collagen and elastic membranes, formation of mesenchymal intimal proliferations, formation of endarteritis verrucosa and swelling and prominence of smooth muscle cells.

Pericardial Sheath Surrounding Pulmonic Root

None of the pulmonic root sections examined from the rheumatic cases was associated with macroscopic pericarditis. In many of the peripulmonic sheaths, however, there were seen scattered lymphocytes and large mononuclear cells with some increase in the number of capillaries. This indicates a low grade irritation phenomenon. All of the active cases presented either a microscopic pericarditis of this type or an acute exudative pericarditis (8 cases) with marked accumulation of inflammatory cells and deposition of fibrin.

Of greater interest, however, were the lesions to be found in the vessels within these peripulmonic sheaths. In a previous publication Gross, Kugel and Epstein[19] have classified and described a large number of lesions affecting the smaller branches of the coronary arteries in the myocardium of rheumatic cases. It will be found that a number of the vascular lesions described as occurring in the pericardial sheaths surrounding the roots of the pulmonary artery and aorta fall into the above mentioned classification. For a detailed description of these lesions the reader is referred to the above mentioned report.

The vascular changes which were found in the control series consisted of: hypertrophy of the media in 3 cases in which the terminal illness was associated with a febrile condition; intimal elastification (this occurred in 2 cases, one individual aged 22 months and one aged 74 years); intimal musculo-elastic hyperplastic changes, with the intimal smooth muscle cells somewhat irregularly arranged (this was found in 1 case, age 9 months); giant medial hypertrophy with

metallaxis (this was found in 2 cases, ages 11 and 58 years respectively). Considered as a whole, some form of vascular lesion was found in the pericardial sheath surrounding the pulmonic root in 18 per cent of these control cases.

In the inactive rheumatic series hypertrophied vessels were found in 15 per cent of the cases; intimal elastification in 1 case; typical intimal musculo-elastic hyperplastic changes in 4 cases (12 per cent) (Fig. 4); and giant hypertrophy with metallaxis in 6 cases (18 per cent). There is thus a somewhat increased incidence over the controls in the occurrence of intimal musculo-elastic hyperplastic lesions and giant hypertrophy with metallaxis. Considered as a whole, some form of vascular lesion was found in the pericardial sheath surrounding the pulmonic root in 33 per cent of the inactive cases. In the active cases the vascular lesions were far more striking. Thus, 60 per cent of the cases showed hypertrophy of the media, 18 per cent intimal musculo-elastic hyperplastic lesions, 34 per cent giant hypertrophy with metallaxis, and 18 per cent intimal fibrosis. Besides these, in 1 case there was present a necrotizing arteritis, and in 1 case (age 11 years) intimal elastification. Considered as a whole, therefore, vascular lesions were found in the pericardial sheath surrounding the pulmonic root in 66 per cent of the active cases.

Summarizing these findings in the peripulmonic sheath, it appears that inactive rheumatic cases show vascular lesions in 33 per cent of the cases, and active rheumatic cases in 66 per cent, as compared to control cases where the incidence was 18 per cent. In the inactive rheumatic group there is a moderately increased incidence of intimal musculo-elastic hyperplastic lesions and of giant hypertrophy with metallaxis; in the active rheumatic cases there is a very decided increase in the incidence of these lesions as well as of intimal fibrosis and medial hypertrophy. Exudative and necrotizing arteritis occurred in 1 case.

Aortic Root Proper

Although the outer third of the aortic media is believed to be supplied with nutrient vessels, very rare capillaries, generally confined to the medial-adventitial border, were observed in only 25 per cent of the aortic roots in the control series. In only 1 case (a child of 9 months with hilar and meningeal tuberculosis) was there present scarring of the media. This consisted of oval and flame-shaped

fibrotic lesions distributed in the middle third of the media. The elastic membranes were ruptured in the scarred areas but no inflammatory cells were present. In 1 case the sparse capillaries were surrounded by scattered lymphocytes and larger mononuclear cells.

In contrast to this the inactive rheumatic cases showed an aortic root medial capillarization in 80 per cent of the cases. In about one-third of these the capillaries were sparse, delicate and confined to the medial-adventitial border. In the remaining cases they were more numerous and penetrated deeper into the media. In these cases the vessels were generally wider in diameter and possessed a rather heavy basement membrane.

Furthermore, in 50 per cent of the inactive rheumatic cases definite scarring of the aortic root media was present. These scars were generally of four types, each more or less representing various grades of damage. A very frequent form of scarring is the flame-shaped lesion. This may be very inconspicuous, avascular and merge imperceptibly with the collagenic interdigitations of the adventitia with the media. In the definite flame-shaped scar the medial elastic lamellae are missing, capillaries are generally present and sometimes a few inflammatory cells. This scar is generally found in the outermost layers of the media, although it can occur in the middle and even inner third.

An equally frequent, decidedly more conspicuous and clear-cut lesion is the oval scar (Fig. 5). This is generally larger than the flame-shaped variety. It is oval in shape with its long axis parallel with the lumen of the aorta. It may be represented by an avascular collagenous area, free of elastic fibers. However, it generally contains capillaries, sometimes arterioles, not infrequently with swollen endothelial cells and occasionally perivascular inflammatory cells. Like the flame-shaped scar it is usually situated in the outer third of the media. This lesion may become so large as to form irregular, patchy fibrotic zones in the outer and even middle third of the media.

A very frequent and characteristic form of scarring is the moth-eaten variety (Fig. 6). This is a somewhat irregular, generally small scarred area with rather inconspicuous collagen. Its most characteristic feature is brought out by staining for elastic tissue. In such preparations it is seen that the more or less regularly ar-

ranged membranes are interrupted in patches which can be described best as moth-eaten areas. These occur generally in the outer third of the media and may be avascular and acellular. They may imperceptibly merge with what is termed the flame-shaped scar. In the latter, however, collagenous tissue is more prominent, the elastica rupture is striking and disorientation of the elastic membranes is more likely to occur.

The least frequent but most marked forms of scarring are the large irregular scars (Fig. 7). These are very obviously distorted areas of the media, often the middle third, with large irregular zones of collagenous tissue, ruptured and distorted elastic membranes and scattered inflammatory cells. They lack, however, gummatous necrosis, giant cells, conspicuous vascularization, marked perivascular lymphocytic and plasma cell infiltrations and the adventitial vascular and perivascular changes characteristic of lues. In the inactive rheumatic material 4 cases showed large irregular scars.

As mentioned before, whereas some of these forms of scarring were avascular, most of them possessed capillaries and even arterioles. The endothelium of these vessels was not infrequently swollen. There was often present a scattering of lymphocytes, large monocytes, sometimes with basophilic cytoplasm and irregular edges, and rare wandering cells of unknown type.

The aortic root media was capillarized in 85 per cent of the active rheumatic cases. In one-fourth of these the capillaries were delicate, inconspicuous, and situated at the medial-adventitial border. In the remaining cases they were of larger caliber, often with heavy basement membranes and swollen endothelial cells. Not infrequently they were associated with or replaced by arterioles. All these cases showed definite scarring. Thus, flame-shaped, oval and moth-eaten scarring occurred in approximately 40 per cent of the capillarized cases, and in 4 cases there were present large irregular scars of such dimensions as to suggest a luetic lesion.

Whereas acute exudative and destructive phenomena were absent in the inactive rheumatic cases, and inflammatory cells were sparse and confined to perivascular sites, the active rheumatic cases showed an astonishingly high incidence of these lesions. Thus, in 20 per cent of the cases, quite apart from the perivascular cellular accumulations (seen in most scarred areas), there was present a distinct inflammatory lesion of various grades of intensity often toward the inner

aspect of the media (Fig. 8) and at times irregularly situated. In a number of cases this lesion was mild and consisted of a sparse and diffuse scattering of ameboid cells with lobated nuclei, orientated very irregularly. These cells were generally of the polymorphonuclear leukocytic variety; some were larger.

In 4 cases the lesion was much more severe. In these there were present eosinophilic swelling of collagen and elastic lamellae, and development of large basophilic cells with irregular edges. These cells were found in the perivascular connective tissue of the scarred areas and also occurred conspicuously in rows between the swollen elastic lamellae (palisades).

Other cellular elements in the inflamed areas were neutrophilic polymorphonuclear leukocytes, eosinophiles, lymphocytes, large monocytes and plasma cells. At times, fragments of eosinophilic collagen were surrounded by solid-staining, irregular, elongated cells which appeared to be made up entirely of nuclear material.

Apart from these inflammatory phenomena a verrucous endarteritis was seen in 1 case. Large conspicuous rows of medial cells were seen in 10 cases, generally associated with other active inflammatory phenomena, and edema was noted in most of the acutely inflamed aortic roots. Intimal reduplications were seen in one-third of the cases. Of these, 3 showed fresh reduplications of the undifferentiated mesenchymal cell variety. Two of these possessed elastic membrane laminations (Fig. 9). In another case the intimal reduplication consisted of fibroblastic tissue permeated with capillaries, which gave it a spongy appearance (Fig. 10).

Summarizing these findings in the aortic root proper it appears that rheumatic fever produces an amazingly high incidence of capillarization, scarring and elastica disruption. The latter lesions occur in four histologically recognizable forms, *viz.* flame-shaped, oval, moth-eaten and large irregular scars. These not infrequently possess capillaries and even arterioles with swollen endothelium. In the inactive cases sparse accumulations of mononuclear cells often occur in the vicinity of the scars, but acute, exudative and destructive phenomena are absent. In 20 per cent of the active cases, however, apart from these perivascular cellular accumulations there were found acute inflammatory phenomena of various grades of intensity similar to those described as occurring in the root of the pulmonary artery. The severity of the lesions was, however, frequently con-

siderably greater than in the latter and the severe lesions occurred much more often. In 1 case the intimal fibroblastic tissue reduplication was permeated with capillaries.

Pericardial Sheath Surrounding Aortic Root

Since most of the periaortic sheaths, particularly in the active rheumatic series, were the seat of a pericarditis, past or present, the adventitial layer was frequently quite dense because of concentration of the organized scar tissue. The adventitial layer not infrequently showed inflammatory cells of the acute, subacute and chronic variety. However, no Aschoff bodies were found in the adventitia proper. Aschoff cells and nodules were observed by Pappenheimer and VonGlahn in 5.4 per cent of 76 rheumatic cases.

The vascular lesions found in the pericardial sheath surrounding the aortic root in the control series consisted of medial hypertrophy in 2 cases, intimal elastification in 1 case and fibro-elastification in 2 cases. Considered as a whole, the pericardial sheath surrounding the aortic root presented vascular lesions in 5 cases (10 per cent) of the control series. In the inactive rheumatic series the incidence of vascular lesions in the pericardial sheath of the aorta was considerably increased. Thus, medial hypertrophy was found in 10 cases, intimal fibrosis in 2, fibro-elastification in 3, typical intimal musculo-elastic hyperplastic lesions in 7, and giant medial hypertrophy with metallaxis in 2 cases. Considered as a whole, the pericardial sheath surrounding the aortic root presented vascular lesions in 17 cases (50 per cent) of the inactive rheumatic series.

Even more striking were the vascular lesions found in the sheath around the aortic root in the active rheumatic series. Thus, medial hypertrophy was found in 33 cases (50 per cent), intimal elastification in 5, fibro-elastification in 2, typical intimal musculo-elastic hyperplastic lesions in 17 (26 per cent), giant medial hypertrophy with metallaxis in 10 (15 per cent), intimal fibrosis in 8, and granular plugged vessels in 1 case. Considered as a whole, the pericardial sheath surrounding the aortic root presented vascular lesions in 44 cases (66 per cent) of the active rheumatic series.

Summarizing these findings in the periaortic sheath, it appears that inactive rheumatic cases show vascular lesions in 50 per cent of the cases and active rheumatic cases in 66 per cent, as compared to

control cases where the incidence was 10 per cent. In the inactive group the incidence of medial hypertrophy and intimal musculo-elastic hyperplastic lesions is significantly high. In the active group medial hypertrophy, intimal musculo-elastic hyperplastic lesions, giant medial hypertrophy with metallaxis and intimal fibrosis occur in a strikingly high per cent of the cases. Granular plugged vessels were found in 1 case.

Discussion

The findings presented in this report appear to be of interest for several reasons. First, they represent another contribution to our knowledge of the damage wrought by rheumatic fever in the first portion of the great vessels, sites which have been on the whole somewhat neglected by previous observers. Secondly, when present, many of the lesions described assume considerable significance when one attempts to determine the nature of various forms of valvulitis in which the inflammatory phenomena are indolent or inactive. In such instances it is not infrequently difficult to decide whether one is dealing with the end result of well known forms of endocarditis, particularly of the rheumatic variety, or whether the lesions represent a type of endocarditis *sui generis* of different etiology. In such studies it is of considerable value to search for other evidence implicating rheumatic fever. While the lesions described in this report are in themselves for the most part not specific, they nevertheless afford additional evidence on which to classify such material either as rheumatic, or in the absence of these changes, to suspect another etiology. Finally, this report serves as an additional contribution to the usefulness of the standardized method of studying hearts histologically which has already been shown to present a high incidence of pathological lesions when present.

One of the most striking findings presented in this report is the unexpectedly high incidence of capillarization, both of the pulmonic and aortic roots. That this occurs in 70 per cent and more of inactive as well as active cases, as compared to 25 per cent of the control non-rheumatic series, and that not infrequently the capillaries are larger, more irregular and of wider distribution in the rheumatic series, opens up many fields for speculation. For example, it seemed of interest to determine if the roots of these vessels in cases of luetic aortitis (particularly those involving the aortic root) presented sim-

ilar findings. An examination of 35 such cases disclosed obvious luetic lesions of the aortic media with marked scarring and disruption of the elastica, gummatous necrosis, vascularization, infiltration with lymphocytes and plasma cells and advanced endarteritic changes with perivascular mononuclear collections in the aortic mantle (chiefly adventitial). These findings, as Klotz has already pointed out, easily differentiate the luetic from the rheumatic lesions. On the other hand, in these same luetic cases the pulmonic root showed surprisingly little involvement. Capillarization and scarring were on the whole somewhat less marked than in the rheumatic series. Furthermore, when vascular lesions were present in the pulmonic sheath they were generally quite different, consisting usually of marked medial hypertrophy with exaggerated intimal fibrosis. While there was noted at times vascular change resembling the intimal musculo-elastic hyperplastic lesion found in the rheumatic series, there was little to be seen of the diversity of vascular lesions observed in the latter. It appears, therefore, that both the very florid inflammatory and destructive phenomena in the aortic root, together with the somewhat different findings in the pulmonic root and its pericardial sheath, serve to differentiate histologically lues from rheumatic fever. It has been mentioned that all of the active rheumatic cases presented in the peripulmonic sheaths either a microscopic pericarditis or an acute exudative pericarditis and that in the inactive rheumatic series many cases showed scattered lymphocytes and large mononuclear cells in this area. It seems possible that this inflammatory condition of the pericardial mantle bears a causal relation to the markedly increased capillarization of the great vessel roots. At any rate, this mechanism must be considered as a factor in addition to spread of the irritative agent by way of the vasa vasorum (Klotz) and by way of the main blood stream (Pappenheimer and VonGlahn).

While scarring is not a conspicuous feature in the pulmonic root in rheumatic fever it becomes extremely important in the aortic root where, as has been indicated by other authors, the lesions may be so extensive as to be confused with lues. In such instances the damage may undoubtedly express itself functionally by producing diminished resiliency and elasticity of the great vessel roots. The smaller lesions, however, appear to be only of histological interest.

The finding of inflammatory lesions in 20 per cent of aortic roots in the active rheumatic series was not unexpected even though the 6 per cent incidence of intimal verrucous lesions was certainly more than anticipated from gross observations. On the other hand, the 14 per cent incidence of inflammatory lesions in the pulmonic root, with 1 case showing a verrucous lesion of the intima, was surprisingly high and of considerable interest.

The intimal reduplications are important histologically only when they are quite fresh, and serve to indicate the presence of activity. The older fibrous reduplications are also found in the control nonrheumatic series.

The vascular lesions in the peripulmonic and periaortic mantles are similar to those described by Gross, Kugel and Epstein. The considerable increase in these lesions both in active as well as inactive cases is in keeping with the findings in the coronary ramifications within the myocardium proper. It is to be noted that, as in the latter, the lesions referred to as intimal musculo-elastic hyperplastic changes, giant medial hypertrophy with metallaxis and intimal fibrosis are the most significant because of their rarity in control nonrheumatic material and because of their conspicuous increase in the rheumatic series. Arteritis and granular plugged vessels were found in only 1 case each.

There remains to discuss the fate of the lesions. This question was taken up in some detail in the above mentioned report by Gross, Kugel and Epstein on the lesions of the coronary arteries and their branches in rheumatic fever, which concerned itself with the myocardial coronary ramifications. It may be said in brief that the somewhat lower incidence of the more characteristic lesions in the pulmonic and aortic roots of the inactive as compared to the active rheumatic fever series indicates that some of them may heal with little discernible residua, some may become transformed into the less characteristic alterations seen in the normal control series due to age period changes, and that the more marked lesions probably occur in patients so violently afflicted with the disease that many fail to reach the inactive stages.

Summary

A variety of lesions found in rheumatic fever in the roots of the pulmonary artery and aorta together with their pericardial mantles

have been described and considered statistically. The observations were made on 66 active rheumatic hearts, 34 inactive rheumatic hearts and 50 non-rheumatic control hearts. It is shown that in both active and inactive rheumatic fever the aortic and pulmonic roots display a strikingly high incidence of destructive and inflammatory lesions consisting of scarring, elastica disruption, vascularization and other inflammatory phenomena. It is also shown that the pericardial mantles surrounding the roots of these great vessels display a high incidence of various vascular lesions. Some of these are similar to those occurring in non-rheumatic controls due to age period changes. Others, however, are similar to the vascular lesions due to rheumatic fever found in the myocardial coronary ramifications. A discussion is given of the significance of these findings.

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DESCRIPTION OF PLATES

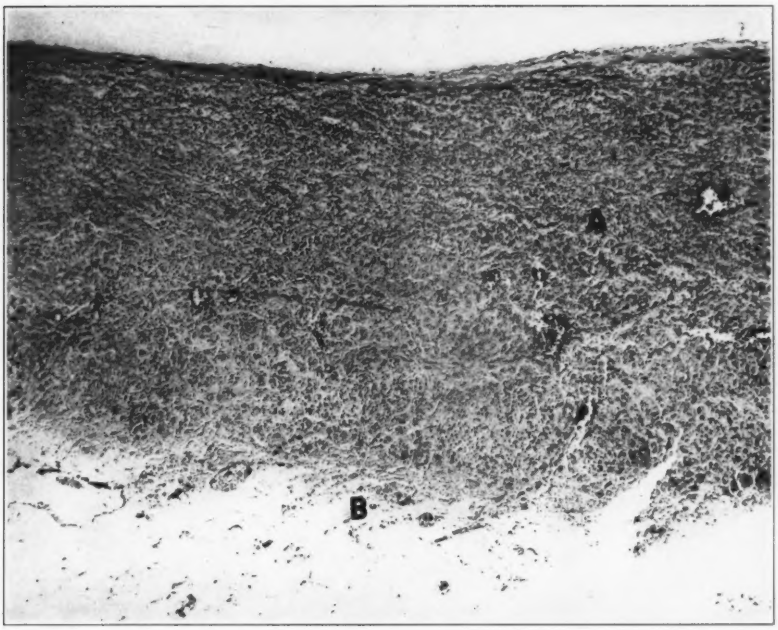
PLATE 84

FIG. 1. Pulmonary artery root from inactive case of rheumatic fever. Age 65 years. Medium power. Hematoxylin and eosin stain.

A = media showing extensive capillarization with penetration into the inner third of the vessel wall; B = adventitia.

FIG. 2. Pulmonary artery root from active case of rheumatic fever. Age 25 years. Medium power. Weigert's elastic and Van Gieson's connective tissue stain.

A = inner zone of pulmonary artery; B = marked elastica destruction, edema, capillarization and infiltration with inflammatory cells; C = adventitia showing mononuclear cell infiltration.



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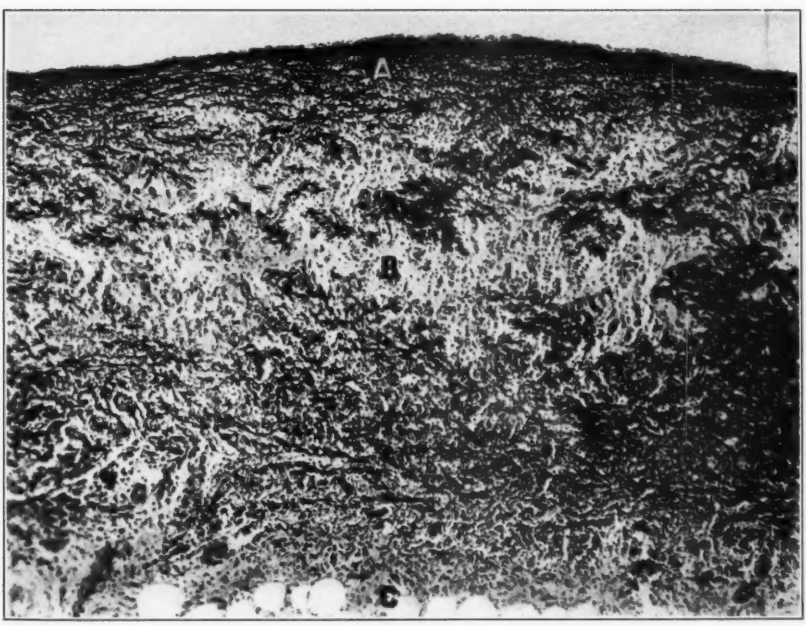

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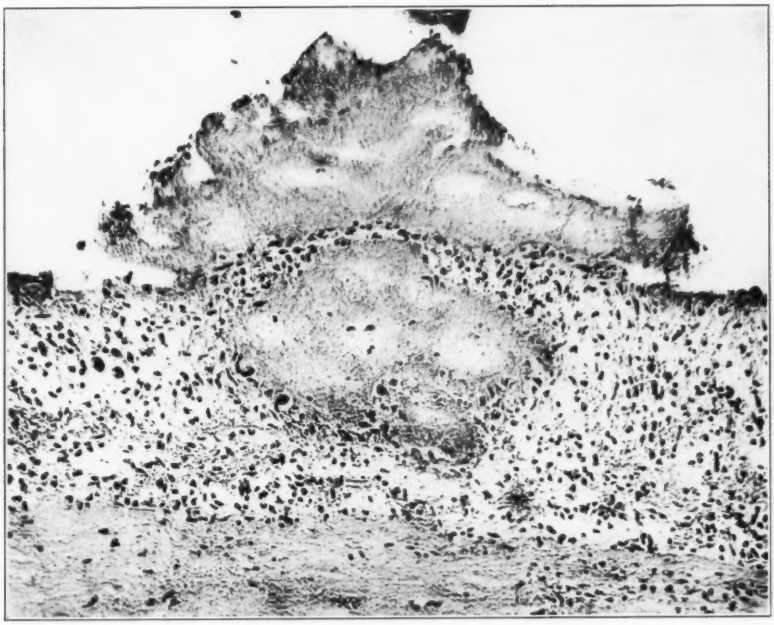
Plate 85

Fig. 3. Pulmonary artery root from active case of rheumatic fever. Age 25 years. High power. Hematoxylin and eosin stain.

Typical verrucous formation with mononuclear cell infiltration at its base.

Fig. 4. Pulmonary artery root from inactive case of rheumatic fever. Age 37 years. Medium power. Weigert's elastic and Van Gieson's connective tissue stain.

A = media; B = adventitia showing two arteries with typical intimal musculo-elastic hyperplastic changes.



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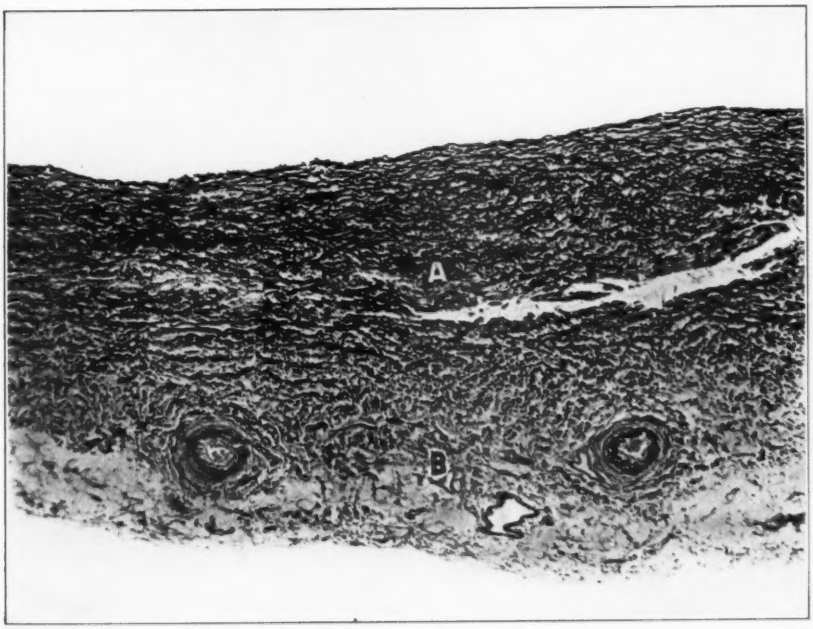

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PLATE 86

FIG. 5. Aortic root from inactive case of rheumatic fever. Age 14 years. Medium power. Weigert's elastic and Van Gieson's connective tissue stain.

A = inner zone of media; B = adventitia. The arrows point to typical oval scars.

FIG. 6. Aortic root media from inactive case of rheumatic fever. Age 34 years. Medium power. Weigert's elastic and Van Gieson's connective tissue stain.

A = inner zone of media; B = outer zone of media. The arrow points to a typical moth-eaten scar.

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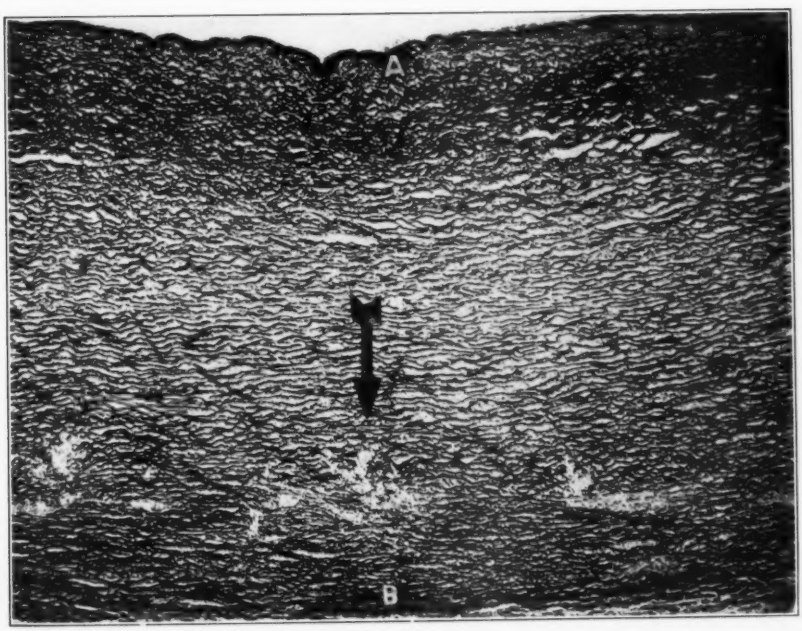

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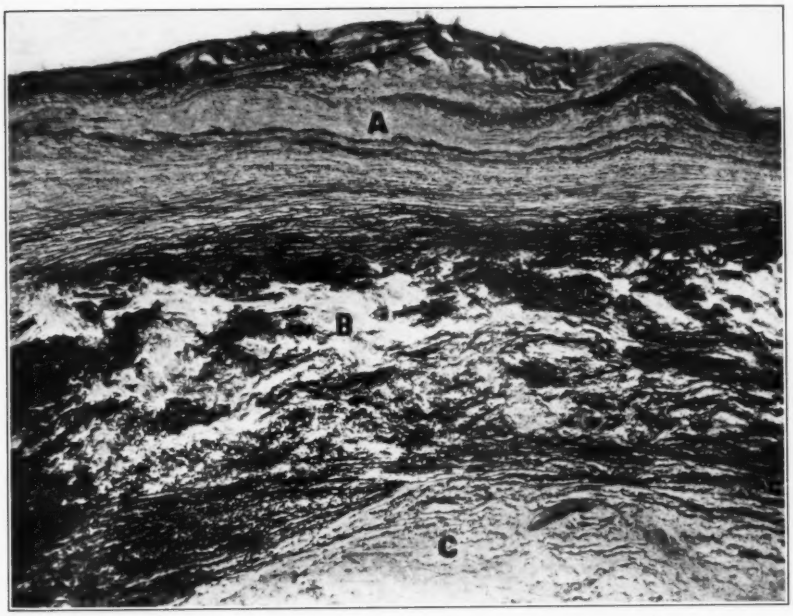
PLATE 87

FIG. 7. Aortic root from inactive case of rheumatic fever. Age 21 years. Medium power. Weigert's elastic and Van Gieson's connective tissue stain.

A = elastic hyperplastic and fibrotic intima; B = large irregular scarring of media with elastica disruption; C = adventitia.

FIG. 8. Intima and inner medial zone of aortic root from a case of active rheumatic fever. Age 25 years. High power. Hematoxylin and eosin stain.

A = intima and subintima showing infiltration with lymphocytes and ameboid polymorphonuclear leukocytes; B = fibrosis of inner medial layer.



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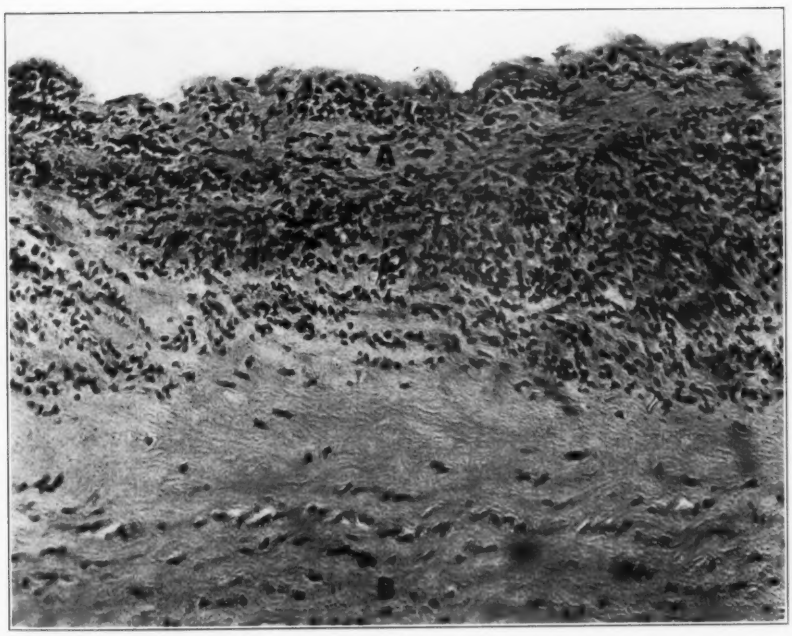

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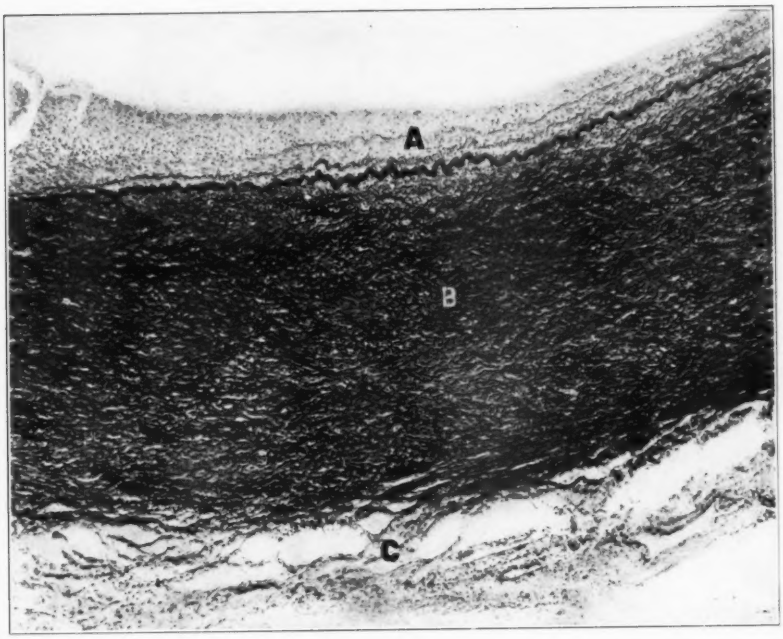
PLATE 88

FIG. 9. Aortic root from active case of rheumatic fever. Age 7 years. Medium power. Weigert's elastic and Van Gieson's connective tissue stain.

A = intimal reduplications showing elastic lamellations; B = media; C = adventitia showing mild lymphocytic infiltration.

FIG. 10. Aortic root from active case of rheumatic fever. Age 13 years. Medium power. Hematoxylin and eosin stain.

A = intimal reduplication converted into spongy mass by an interlacing network of capillaries within a fibroblastic matrix; B = media with scattered lymphocytes and polymorphonuclear leukocytes; C = adventitia with mild lymphocytic infiltration.



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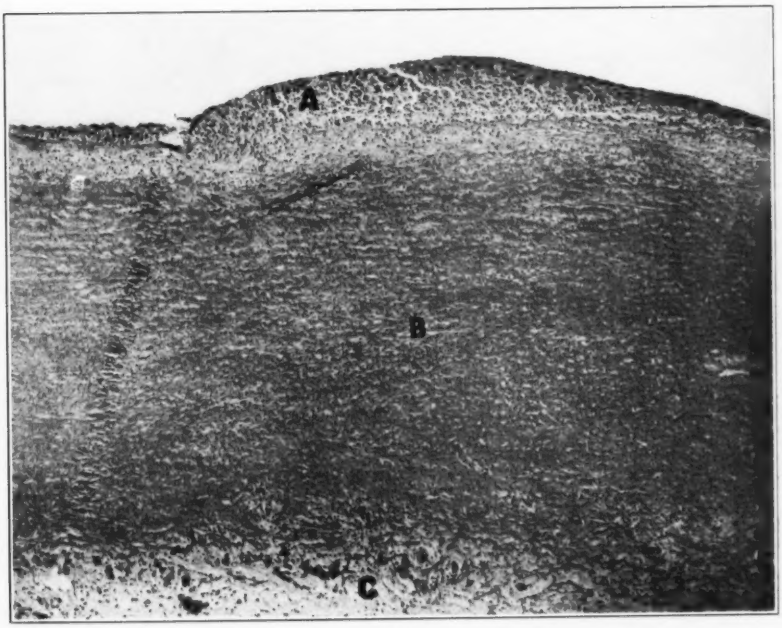

10

NUCLEAR INCLUSIONS SUGGESTIVE OF VIRUS ACTION IN THE SALIVARY GLANDS OF THE MONKEY, *CEBUS FATUELLUS* *

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Evidence is fast accumulating that we must recognize a special group of salivary gland viruses. All of them have been discovered by chance. They are so benign that attention was not directed to them by distinctive clinical symptoms. What attracted notice was the extraordinary hypertrophy of certain acinous or duct cells accompanied by the formation in their nuclei of inclusions resembling those caused by viruses.

The first inclusion-laden cells were reported under the heading of "protozoan-like bodies" in the parotids of two infants by Ribbert [1] and in the submaxillary glands of guinea pigs by Jackson.[2] Credit is due to Goodpasture and Talbot [3] for recognizing the close resemblance between the bodies in humans and guinea pigs and for pointing out the similarity of both to the intranuclear inclusions described by Tyzzer [4] in varicella. Lipschütz [5] then rediscovered the intranuclear inclusions in herpes, admirably described and illustrated by Kopytowski,[6] and emphasized the great importance of these bodies in "inclusion diseases" in general. But it was Kuttner and Cole [7] and Kuttner [8] who led in the demonstration that the inclusions in guinea pigs are actually caused by a virus. This naturally excited so much interest that investigators, while examining the salivary glands of other animals, have been on the lookout for nuclear inclusions, with the result that at the present time they have been reported in rats,[9] moles,[10, 11] mice [12] and hamsters.[13] Finally Kuttner and Wang [13] have proved that the intranuclear inclusions in hamsters, mice and wild rats are caused by viruses that are very similar to the submaxillary gland virus of guinea pigs. We await with interest proof of virus action in the formation of the inclusions in humans and moles.

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Observations

As in the discovery of the intranuclear inclusions in the other forms mentioned, so also in *Cebus fatuellus* they were encountered almost by chance.[14] A routine examination was being made of all the principal tissues of 2 *Cebus fatuellus* and 18 *Macacus rhesus* monkeys which received repeated doses of irradiated ergosterol. The inclusions were seen in the parotid and submaxillary glands of both of the Cebus monkeys but in none of the Macacus. Their appearance is illustrated in the plate.

All of the inclusion-laden cells were so much hypertrophied that they could easily be recognized without the use of an oil immersion objective. What usually caught the eye was a large, spherical, eosin-staining mass — the nuclear inclusion — with a clear halo between it and the nuclear membrane. The cytoplasm of the affected cells was distinctly basophilic, owing to the presence of cytoplasmic bodies, and consequently stood out sharply against the neighboring cells which were acidophilic, for the inclusions were limited to the secretory and intercalated ducts. None were found in the secretory acini. The altered cells were spherical in shape and bulged so far into the lumen that in some cases they seemed to occlude it.

Though easily seen, the inclusions were difficult to find by reason of their extreme rarity. A total of 33 was studied; of these 11 were seen in 102 sections of the parotid and 22 in 149 sections of the submaxillary gland. Each section was 7μ thick and about 5 mm. square. The largest number of inclusions ever seen in a single section was 4. After about a month spent in their study they could be found at the rate of 4 or 5 per hour. No inclusions were detected in the sublingual glands.

The size, and indeed all of the structural features, of the inclusions and of the cells containing them were remarkably uniform. The cells, nuclei and inclusions were roughly spherical. The diameters of 30 cells and nuclei and of 29 nuclear inclusions were measured in microns (Table I).

In the case of a binucleated cell the maximum diameter amounted to 25.2 μ, but the size of the nuclei and their inclusions remained about the same. Traces of the second nucleus in the binucleated cell illustrated in Figure 6 are seen below and to the left.

All of the nuclear inclusions were strongly acidophilic when viewed

after fixation in formalin-Zenker and coloration with hematoxylin and eosin. They contained no hematoxylin-staining material. When cut through the middle they appeared to be optically homogeneous, but thin slices of their surface showed them to be made up of tiny, acidophilic adherent particles. The outlines of the inclusions were usually quite sharp. Only 1 inclusion was observed per nucleus.

The halo interposed between the inclusion and the nuclear membrane was altogether free of basophilic chromatin near the inclusion, but as one approached the membrane some particles of basophilic

TABLE I

Measurements of Cells, Nuclei and Nuclear Inclusions

Diameters	Cells	Nuclei	Inclusions
Maximum largest diameter	19.2	13.2	7.2
Minimum largest diameter	12.0	8.4	3.6
Average largest diameter	15.9	13.2	7.2

material could be made out — in other words, margination on the nuclear membrane was not usually complete (see Figs. 5 and 6). The width of the halo about the inclusion was approximately the same on all sides.

Nucleoli were identified in all instances where the section included a large amount of nuclear material. Invariably the nucleoli, one or two in each nucleus, left the central nuclear zone and became closely applied to the nuclear membrane. The uniformity in this migration of nucleoli, in their size (approximately 1.5 by 3μ), degree of flattening and staining reaction was quite striking. Such a marginated nucleolus is illustrated to the left side of the nuclear inclusion in Figure 3 and above it in Figures 4 and 5. The coloration indicated an even mixture of acidophilic and basophilic material, the latter almost masking the former.

Cytoplasmic inclusions were observed in 21 out of 33 nuclear inclusion-laden cells and are represented in Figures 2 to 6. They were distinctly basophilic and most of them were particulate in consistence. The largest exhibited a maximum diameter of 6μ and a minimum of 3.6μ; the smallest was a sphere about 1.5μ in diameter and the average, likewise spherical, was approximately 2.5μ in diameter.

These cytoplasmic inclusions occurred in the distal cytoplasm between the nucleus and the lumen of the duct. Most of them were not provided with clear halos. Some, which were of much denser consistence, did have halos.

The epithelial cells next to those in which inclusions had developed showed no particular sign of injury, revealed by the simple technique mentioned, except that they were mechanically pushed aside. It may be that they were very slightly increased in size, as Scott and Pruett [15] found by careful direct measurements to be the case in the submaxillaries of guinea pigs. The intercalated ducts showed no evident alterations, but the secretory ducts, which contained many more cells and in which most of the inclusions were found, did look abnormal. The cells in which nuclear inclusions had not formed did not all present the same appearance. None showed evidence of hydropic degeneration, as illustrated by Pearson [16] in his Figure 12. Some were nevertheless singled out from the rest by the intense acidophilia of their cytoplasm and the pyknosis of their nuclei. Both cytoplasm and nucleus were shrunken. Such cells were observed alone and in groups, but in number they were always less than 50 per cent of the total. The lumen of one secretory duct was crowded with them. If this degeneration and desquamation of duct cells were a normal process, one would expect multiplication of the remaining cells to make good the loss, but no mitoses were seen.

Since the salivary glands of other Cebus monkeys, which had not received irradiated ergosterol, were not available for comparison, the best that could be done was to shift to similar tissues of *Macacus rhesus*. All of 18 given the irradiated ergosterol in equal and sometimes greater amounts exhibited the same process of degeneration and desquamation but none of them to the same degree. It was also seen in 4 untreated Macacus monkeys. Although these degenerating cells never showed any tendency whatever to inclusion formation, the possibility remains that their presence in such numbers is an accentuation of a normal process of elimination caused by injury to the duct epithelium and related in some way to the formation of nuclear inclusions, despite lack of topographical association between the two. We do not, however, advance this as a suggestion.

Another change in the ducts which should be reported, but not used as evidence, was accumulation of fluid about the bases (proximal poles of the cells) so that they were often quite widely separated

from the usually contiguous acinous tissue. This, also, was less marked in the Macacus monkeys. Calcium concretions occurred in both Cebus and Macacus, but were more numerous and larger in the former. They were probably caused or intensified by the irradiated ergosterol, as will be described in a later paper by Cowdry, Scott and Möller.[17] Proliferation of fibroblasts and slight leukocytic infiltration took place near them, but the nuclear inclusions were not unusually abundant in their vicinity. Lymphocytic infiltration was often seen in the Cebus monkeys and less frequently in the Macacus. Of the 33 inclusion-containing cells in the Cebus, 20 were located in areas of this sort of infiltration. On the other hand, their presence in such areas was the exception rather than the rule because they were so rare and the invading lymphocytes so numerous.

The following tissues, additional to the salivary glands, were examined in each of the 2 Cebus monkeys to ascertain how widespread the formation of nuclear inclusions might be in the organism as a whole:

Fundus, ileum, colon, duodenum, pancreas, spleen, liver, adrenal, kidney, urinary bladder, lymph node, skeletal muscle, lung, thymus, thyroid, parathyroid, trachea, esophagus, mucous membrane of cheek, sublingual glands, testis, prostate, jejunum, pituitary, skin of back, heart muscle and bone marrow.

Since no nuclear inclusions at all like those in the submaxillaries and parotids were seen, it is evident that the reaction was restricted to these glands. Rare acidophilic droplets in the nuclei of the acinous cells of the pancreas of 1 monkey and of the pars distalis cells of the pituitary of the other were disregarded. The animals were quite mature, for their long bones had become ossified and their testicles showed active spermatogenesis. They weighed 1510 gm. and 1650 gm., respectively. Each received 6 daily doses of 5 cc. irradiated ergosterol 10,000 x (Mead Johnson and Company). All except the first of these were given with 10 gm. of calcium gluconate in tomato juice. They were killed 3 days after the last dose, at which time the blood calcium and phosphorus of 1 was 9.7 and 9.9, and of the other 9.6 and 5.

Attempts to determine if these salivary gland inclusions are caused by a virus were not undertaken because the inclusions were not discovered until weeks after the animals had been killed. Having in mind the results of transmission experiments with other salivary gland viruses, the chances of success would be good only if the

ground up glands of adults possessing inclusions were injected into animals of the same species so young that they had not acquired a natural infection and were, therefore, susceptible.

The nuclear inclusions in these Cebus monkeys did, however, exhibit a close resemblance to those known to be caused by viruses in the salivary glands of the guinea pig, rat, mouse and hamster. Like inclusions in the same location of moles and humans, it can only be said that their presence suggests the possible action of a virus. The nuclear inclusions in moles were more basophilic, but we found that this basophilia was at least partly due to failure of the basophilic chromatin to lose its basophilic properties or to marginate on the nuclear membrane. Instead of doing so, it accumulated in the form of a thin layer about the inclusion. The coating could easily be seen in favorable specimens. No measurements have been made, but the intensity of acidophilia of the core of the nuclear inclusion inside this basophilic layer in moles may closely approach that of the entire inclusion in Cebus, to which no basophilic material is attached.

In shape the inclusions are more uniformly spherical than the type inclusions in the guinea pig. The latter are often elongated in cells that are flattened. Thus, we have not found in Cebus any departure from the spherical shape of inclusions or cells comparable with that illustrated by Pearson.

The basophilic cytoplasmic inclusions in Cebus exhibited a greater range in size than in either the guinea pig or the mole. Consequently they are not so frequently disposed in orderly rows between the nucleus and the lumen. After formalin-Zenker fixation and staining with hematoxylin and eosin, the vast majority of them appeared to be made up of tiny uniform particles which look a little like Rickettsiae. These masses of particles were ordinarily not provided with halos. Occasionally, however, an inclusion, much more dense and not visibly particulate, was seen among the others and was limited by a halo. Very rarely all of the cytoplasmic inclusions in a single cell showed this peculiar density plus halos. Clear vacuoles may occur in the inclusions as well as in the cytoplasmic ground substance, but they were rare. When the section was overstained with hematoxylin the cytoplasm was likewise colored blue and the outlines of the inclusions could only be distinguished with difficulty. Structural details were also obscured if the sections were too thick, that is

more than 7μ. The characteristic margination and partial flattening of the nucleolus on the nuclear membrane referred to in the Cebus inclusion-laden cells was not encountered so repeatedly in the mole in which the nucleoli often held their spherical outlines and remained in contact with the inclusions.

Discussion

Evidently *Cebus fatuellus* is to be added to the other species mentioned as exhibiting nuclear inclusions suggestive of the action of a salivary gland virus. The salivary gland viruses discovered in

Table II

Comparison of Salivary Gland Viruses with Pathogenic Viruses

Salivary gland viruses	Pathogenic viruses
No recognizable symptoms	Severe symptoms
Affect infants	Older individuals, infants often relatively immune
Marked cellular hypertrophy	Little or no hypertrophy
Larger inclusions	Smaller inclusions
Accompanied by basophilic cytoplasmic inclusions	Not so accompanied
Inclusion-laden cells persist for months	Are quickly removed
Active virus remains latent in tissue apparently as long as inclusions persist	Soon disappears or loses potency, for transmissions must be made promptly if they are to be successful
Virus has been transmitted only to same species	In many cases it has been transmitted to a wide range of species
Transmission only to individuals so young (generally under 1 month) that they have not become naturally infected	Adults equally satisfactory for transmission unless previously infected
Incidence in a group high, in some instances 100 per cent	Incidence comparatively rare

guinea pigs, rats, mice and hamsters, and suspected in humans, moles and *Cebus fatuellus*, are probably representatives of quite a large group, for it is likely that many others will be discovered. Even with the evidence now available it is possible to contrast these inapparent salivary gland viruses with pathogenic nuclear inclusion-producing viruses, like those of herpes and yellow fever (Table II).

The association between persistence of inclusions and presence of active virus may be stressed. Our reason for thinking that the inclusion-laden cells persist for months depends upon the age of formation, the age of disappearance and the likelihood of replacement of old inclusions by newly developed ones in the interval.

The age of formation is certainly early. Thus, Löwenstein [18] found inclusions in the parotid of a 2 months old infant; Wagner [19] in the sublingual of a 2 weeks old infant; Cole and Kuttner [20] in 3 out of 43 guinea pigs less than 1 month old; and Thompson [12] in 10 out of 70 rats, 2 months old.

Very little attention has been paid to the age of disappearance. In humans Farber and Wolbach [21] found inclusions in 22 out of 183 infants of 17 months or less. This was a 12 per cent incidence, but 80 per cent were in individuals under 1 year of age. After 1 year they probably disappear quite rapidly, but accurate data on a large series are lacking. In "adult" guinea pigs they have been observed by many workers and in high percentages of 80 or more. It is difficult to estimate the age of adult guinea pigs ordinarily used in the laboratory but it can be taken conservatively as 1 year. Thompson failed to find inclusions in 12 rats 6 months old. Our Cebus monkeys were at least 3 years old but the age of inclusion formation is not known for them or for adult moles. The available information about adult hamsters and mice is not helpful.

With reference to replacement in humans, a statement by Farber and Wolbach is instructive: "One perplexing feature in our study of the inclusion bodies was failure to find small forms which could with confidence be interpreted as stages in formation. If the inclusions were present at all they were strikingly alike and within a narrow range of size and detail." The Rectors [11] in their report on moles also remarked upon the uniformity of the inclusions and the absence of young forms. The same feature was exhibited by Cebus.

It is clear from the experiments of Pearson in our laboratory that cells hypertrophied to the maximum degree and charged with nuclear and cytoplasmic inclusions are dead. The nuclear changes alone are so drastic as to appear incompatible with life. All the epithelial cells of the glands possess mitochondria in the usual number except the inclusion-laden ones from which the mitochondria have completely disappeared. Neither do they behave like living cells. When Pearson transplanted the glands of guinea pigs into the

peritoneal cavity, these cells with inclusions differed sharply from all the rest by not exhibiting autolytic changes; we think, because they were already dead.

Dead cells are as a rule promptly disposed of by the organism. It is surprising how these persist, maintaining their distinctive properties without being desquamated, falling a prey to phagocytes or being removed by autolytic enzymes. It is also interesting that we have come to rely upon such dead cells as marking the existence of active virus.

Is the virus bound to the surface of the dead cells or locked away in their cytoplasm or nucleus? If it is simply a case of adsorption on their plasma membranes the association must be a very intimate one amounting almost to chemical combination for, as pointed out by Scott and Pruett, the cells are so placed that their surfaces are being constantly washed by the passage of large volumes of water. In some species they are in acinous cells, in others in duct cells or in both. The duct cells are of the intercalated and secretory variety. It is by transfer through the walls that water is added to the secretory products. No instance is on record of the formation of inclusions by the cells of the collecting ducts, the walls of which are not regularly flushed by fluid tides in this way. Nature has indeed selected one of the worst places in the body for the attachment of virus to cells if this attachment is capable of being loosened by currents of fluid. On the hypothesis that the virus is held in the cytoplasm or in the nucleus, the immunity of the dead cells to the usual forces of disintegration would appear to favor its persistence over long periods.

Summary

Intranuclear inclusions, closely resembling those caused by salivary gland viruses, were found in the parotid and submaxillary glands of 2 *Cebus fatuellus* which had received irradiated ergosterol but which showed no signs of disease. No inclusions were observed in the salivary glands of 18 *Macacus rhesus* similarly treated.

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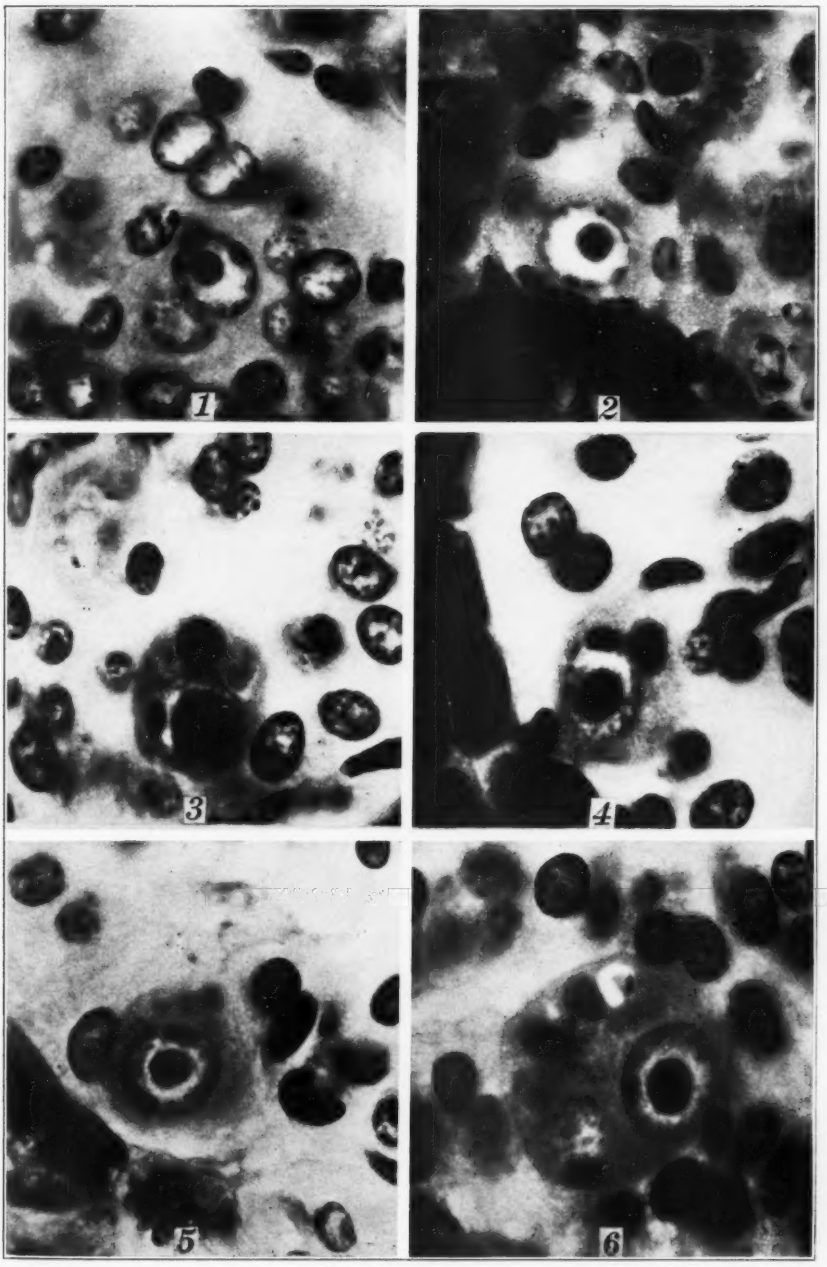
DESCRIPTION OF PLATE

PLATE 89

FIGS. 1–6. Photomicrographs taken at a magnification of 1600 diameters of nuclear inclusion-laden cells in the salivary glands of *Cebus fatuellus*. Figs. 1 and 2, and 5 and 6 are of the parotid, and Figs. 3 and 4 of the submaxillary gland.



Cowdry and Scott Nuclear Inclusions Suggestive of Virus Action

NUCLEAR INCLUSIONS IN THE KIDNEYS OF *MACACUS RHESUS* MONKEYS *

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Evidence is accumulating that the kidneys may be the site of action of inapparent viruses just as the salivary glands have been shown to be. Hypertrophied cells possessed of nuclear inclusions suggestive of virus action have been reported in the kidneys of human fetuses and young children by a number of investigators. Some of the problems raised by their discovery have been critically considered by Farber and Wolbach.[1] Intranuclear inclusions have been noted in the kidneys of London sewer rats and of *Macacus rhesus* and *Cercopithecus* sp. monkeys by Hindle and Stevenson. They have also been found by Cowdry, Lucas and Fox[3] in several other species, likewise without symptoms of disease. In this paper we shall consider the inclusions in *Macacus rhesus* particularly after the administration of large doses of irradiated ergosterol 10,000 x (Mead Johnson and Company).

Observations

We first encountered the inclusions in the kidneys of 12 out of 16 *Macacus rhesus* which had been treated with the irradiated ergosterol. But, instead of being very rare and of exhibiting uniform properties like those in the salivary glands of *Cebus fatuellus*,[4] moles,[5] rats[6] and other forms, these renal nuclear inclusions occurred in large numbers and were highly variable. They could be arranged in series beginning with a normal cell and extending by imperceptible changes to a considerably enlarged cell with hypertrophied nucleus and conspicuous nuclear inclusion (Figs. 1 and 2). That is to say, we seemed to be dealing with a process active at the time the kidneys were fixed. There was no definite tissue reaction about the affected cells. The kidneys of 2 animals exhibited marked lymphocytic infil-

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tration, of 5 slight infiltration, and of the remaining 9 no infiltration. Some of the inclusion-laden cells showed the classical features of cloudy swelling but others did not. Desquamation of cells into the lumen was slight. One kidney exhibited marked calcium deposition but in it the inclusions were not particularly abundant. In the kidneys that showed most inclusions the nuclei of many of the tubule cells were very atypical. Some were enormously enlarged (Figs. 3 and 6), had budded extensively so that a single nucleus was replaced by 20 or more tiny ones (Fig. 5), or contained vacuoles that stained feebly with eosin (Fig. 4). A few nuclei were in process of mitotic division. The picture seemed almost neoplastic but there was no new formation of tubules. Evidence, however, of a relation between this nuclear polymorphism and inclusion formation was not forthcoming. Some of the largest inclusions occurred in nuclei that were enlarged but not atypical in structure and the most hypertrophied and atypical nuclei were sometimes devoid of inclusions (Fig. 6). The maximum weight of the animals showing inclusions was 3800 gm., the minimum, 2560 gm. and the average, 2960.9 gm. The sexes were about equally divided. They were 3 to 4 years old.

Examination of the kidneys of 10 normal controls brought to light only 1 that exhibited nuclear inclusions — an incidence of 10 per cent as compared with 75 per cent in the monkeys treated with irradiated ergosterol. To answer the question of whether or not the irradiated ergosterol was responsible for this increase we looked for nuclear inclusions in all of the kidneys of Macacus available in our laboratory and found them in:

16 out of 85, or 18.8 per cent of experimental poliomyelitis animals, many of which were killed before severe symptoms ensued.

2 out of 4, or 50 per cent found dead of unknown cause.

None out of 18 (acute diarrhea, 9; rabies, 1; meningitis, 1; tuberculosis, 4; pneumonia, 2; and 1 killed after injection of measles blood).

Interpretation of the results of any search of this sort for inclusions will depend upon how comprehensive it was. In this case the survey was limited to a thorough examination, with the aid of a mechanical stage, of one hematoxylin and eosin-stained section from each animal. The sections were 7μ thick and averaged 2 sq. cm. in extent. They passed through cortex and medulla in a direction at right angles to the external (lateral) surface and midway between anterior and posterior poles. About 80 per cent of the sections in-

cluded the renal pelvis. Undoubtedly the incidence of inclusions would have been greater had more tissue been examined. But 18.8 per cent in the poliomyelitis animals and 16.8 per cent in the whole series of 107 is distinctly less than 75 per cent in the monkeys given irradiated ergosterol. The percentage for the latter was calculated on the basis of a comparable examination of a single section from each monkey. The 10 normal monkeys and the 107 employed in different experiments were of about the same size and age as those subjected to irradiated ergosterol.

Further support for the theory that the irradiated ergosterol aided in inclusion formation came from a comparison of the inclusions in animals treated and untreated with ergosterol. Among the 18 positive cases, in the 107 which did not have the ergosterol, 4 contained many inclusions with grading properties indicative of an active process and 14 showed only rare, hypertrophied inclusion-laden cells reminiscent of those in the salivary glands of Cebus, which point, we think, to a static condition. No more than 2 of these large cells were observed in a single section. One was binucleated and 2 were not cut in a plane favorable for making measurements. This left only 13, of which the measurements in microns given in Table I were made.

TABLE I

Measurements of Cells, Nuclei and Inclusions

Measurements	Cells	Nuclei	Inclusions
	μ	μ	μ
Maximum largest diameter	19.2	15.6	7.2
Minimum largest diameter	12.0	9.6	4.5
Average largest diameter	15.0	11.0	5.6

Evidently these measurements were not very different from those already given by us for the inclusion-laden cells in the salivary glands of Cebus.[4] The inclusions were likewise wholly acidophilic. Margination of chromatin and flattening of the nucleolus on the nuclear membrane took place (Fig. 9) in the same way and apparently to the same extent. Stages in the final disintegration of the affected cells were lacking, as in the salivary glands, and cells immediately next to them showed no alterations not seen in more remote ones. Two differences, perhaps of minor importance, were

noted. The nuclei of the affected renal cells were less uniformly spherical and basophilic cytoplasmic inclusions were rarer, being detectable in 4 out of 18 cells as compared with 22 out of 33 in the salivary glands. They are best illustrated above and to the left of the nucleus in Figure 9. The point to be emphasized is that in this large series of non-treated monkeys the inclusion-containing cells in the majority of kidneys showing inclusions (14 out of 18) were all modified to about the same degree, were of rare occurrence and unaccompanied by a distinctive local reaction pointing, as we have intimated, to a process which was latent, halted or static.

A study of the distribution of inclusions was made to ascertain whether there was a significant spreading of the condition in the 12 Macacus given irradiated ergosterol and possessed of inclusions, as compared with the others. No inclusions were seen in the cells of the renal corpuscles or renal pelvis of any of the animals. In Table II the presence of many nuclear inclusions of variable size — our presumed active process — is indicated by a plus sign. When large hypertrophied cells were seen, the number in the section is presented in numerals 1 or 2, for there were never more. When no inclusions were seen a minus sign is inserted.

The data in the table show how much more widespread, as well as more numerous, were the inclusion-holding cells in the animals given irradiated ergosterol, but do not tell the whole story of distribution. The thin and thick segments exhibited inclusions only when situated in the cortex. The parts of these segments extending into the medulla in the Malpighian pyramids, like the collecting tubules in the pyramids, were always devoid of inclusions in this lot of animals as far as our observations went. It was unusual to find inclusions in the cortex immediately within the capsule. The inclusions were principally centered in the cortical substance at a depth from the capsule of more than 100 μ in both types of convoluted tubules, in the thick segment, initial collecting tubules and those parts of the collecting tubules in the medullary rays.

The protocols of the experiments were then studied to discover whether or not a relation existed between the dosage of irradiated ergosterol and the activity of inclusion development. For this purpose the kidneys were listed in groups depending on the number of inclusions. The data given in Table III show no evidence of such relation.

The literature abounds in references to renal lesions caused by various irradiated ergosterol or cholesterol preparations in humans,[7, 8] dogs,[9, 10] rats,[11] rabbits,[12, 13] guinea pigs [13] and other forms, but there is no mention of the formation of nuclear inclusions. While

TABLE II

Distribution of Inclusions

Monkeys	No.	Neck segment	Proximal convoluted tubule with medullary portion	Thin segment medullary loop	Thick segment medullary loop	Distal convoluted tubule	Initial collecting tubule	Collecting tubule	Ducts of Bellini
Treated with irradiated ergosterol	67	−	+	−	+	+	+	−	−
	89	−	+	−	+	+	+	+	−
	113	−	+	+	+	+	+	+	−
	124	−	+	+	+	+	+	+	−
	127	−	+	−	+	+	−	−	−
	134	−	+	−	−	−	−	−	−
	135	−	+	+	+	+	+	−	−
	141	−	−	−	+	+	+	+	−
	143	−	−	−	−	+	−	+	−
	146	−	+	−	+	+	+	+	−
	158	−	−	−	−	−	−	+	−
	358	−	+	−	−	+	−	−	−
Not treated	5	−	+	−	+	+	−	−	−
	10	−	1	−	−	−	−	−	−
	20	−	1	−	−	−	−	−	−
	30	−	+	−	+	+	+	−	−
	31	−	1	−	−	−	−	−	−
	40	−	−	−	−	−	2	−	−
	57	−	−	−	−	1	−	−	−
	62	−	−	−	−	1	−	−	−
	84	−	−	−	−	1	−	−	−
	110	−	−	−	−	1	−	−	−
	111	−	−	−	1	−	−	−	−
	116	−	+	−	+	+	−	−	−
	117	−	−	−	−	1	−	−	−
	119	−	1	−	−	−	−	−	−
	133	−	1	−	−	1	−	−	−
	172	−	−	−	−	−	−	−	+
	189	−	−	−	−	1	−	−	−
	234	1	−	−	−	−	−	−	−

it is possible that the inclusions might occasionally have been overlooked by individuals not actively searching for them, the uniform absence of reports of their occurrence suggests that the administration of irradiated ergosterol is not alone responsible for their development. All that can be said, therefore, is, that in our monkeys

the irradiated ergosterol may have activated or intensified a process already latent in the kidneys, as indicated by the occasional finding of inclusion-laden cells in the untreated monkeys. In the submaxil-

TABLE III

Study of Relation Between Dosage of Irradiated Ergosterol and Activity of Inclusion Development

Inclusions	No.	Total cc. ergosterol	Number of doses	Period in days	Blood calcium	Blood phosphorus
					mg. per 100 cc.	*mg. per 100 cc.*
Inclusions most numerous	89	30.0	27	153	17.1	7.2 2 days before death
	124	5.0	1	4	12.0	..
	146	6.5	13	85	10.5 9 days before death	..
	113	11.0	20	132	9.31 2 days before death	5.6
Very numerous	67	5.0	1	3	..	..
	127	37.5	26	152	11.3	11.0 1 day before death
	135	14.0	21	151	10.7	7.7
	358	13.0	20	265	11.2	5.8
Numerous	141	10.0	2	31	11.5	..
	143	126.0	126	130	14.9 4 days before death	6.5
Rare	134	33.0	28	152	10.8	7.3
	158	33.0	28	152	18.4	8.5
No inclusions	144	5.0	1	5	11.8	..
	147	12.5	20	263	11.3	5.5
	A	30.0	3	73	12.67	8.2 1 day before death
	B	12.0	2	5	12.94	7.5 1 day before death

lary glands of guinea pigs Scott [14] suppressed the development of inclusions by ligation of the duct and greatly increased it by the injection of pilocarpine, thus demonstrating the influence of experi-

mental alterations in physiological activity. The nuclear inclusions found by Pappenheimer and Maechling [15] in kidney cells as the result of the administration of certain bismuth preparations are obviously different from those with which we are concerned on account of their marked basophilia and other features.

A survey was made of other tissues of these Macacus monkeys to determine whether the reaction of nuclear inclusion formation was restricted to the kidneys or not. In the following list of tissues the number of animals in which each was examined is given in parentheses.

Fundus (16), ileum (13), colon (13), duodenum (5), pancreas (15), spleen (15), liver (20), adrenal (15), urinary bladder (17), lymph node (11), skeletal muscle (13), lung (15), thymus (12), thyroid (13), parathyroid (8), trachea (12), esophagus (9), mucous membrane of cheek (8), ovary (8), vagina (4), uterus (7), testis (6), prostate (5), jejunum (9), pituitary (6), skin of back (5), Fallopian tube (2), heart muscle (24), cerebral cortex (4), cerebellar cortex (6), cervical spinal cord (5), cervical spinal ganglia (3), bone marrow (5), lacrimal gland (1), tonsil (2), mammary glands (2), submaxillary gland (13), parotid (11) and sublingual gland (1).

No greatly hypertrophied, inclusion-holding cells comparable to those which we have reported in the salivary glands of Cebus and in the kidneys of Macacus were encountered, but we did find nuclear inclusions of Cowdry's type B [16] in the medullary cells of 11 adrenals, in hepatic cells of 4 livers, in epithelial cells of 1 lung and in the pars distalis cells of 1 pituitary. In all these tissues the inclusions were small, the cells carrying them appeared in other respects to be normal and they were of rare occurrence, although no single section of the tissues mentioned was absolutely free from them.

It will be recalled that Stewart and Rhoads [17] and Covell [18] have reported nuclear inclusions possibly caused by a virus in *Macacus rhesus* in the absence of clinical signs of disease. Covell discovered them in either the nasal mucous membrane, trachea, bronchioles, alveoli of lungs or bile ducts of the liver in 20 out of 60 monkeys. The highest frequency was in the pulmonary alveoli of 12 animals. He stated that no inclusions were observed in other parts of the body. The inclusions which he figured do not look much like those with which we are concerned. In the absence of transmission experiments it is idle to speculate on the relation of the inclusions we have reported in the kidneys to the others we have found elsewhere and to those of Stewart and Rhoads and of Covell.

Discussion

The nuclear inclusions in the series of *Macacus rhesus* not treated with irradiated ergosterol closely resemble those that we have found in the salivary glands of *Cebus fatuellus*. In both there are: (1) great nuclear hypertrophy; (2) development of single, spherical nuclear inclusions that are acidophilic; (3) flattening of nucleolus on nuclear membrane; (4) partial or total margination of all basophilic chromatin on the nuclear membrane so that the inclusion is surrounded by a distinct and wide halo; and (5) the formation of basophilic, cytoplasmic inclusions. There is the further common feature of uniformity and rarity of the inclusion-laden cells, pointing, we believe, to a latent rather than an active process in both kidneys and salivary glands for reasons already presented.[4] This close resemblance is compatible with the view that the nuclear inclusions in both situations are produced by a single or by two closely related viruses. In favor of the idea that one virus is acting in human infants, is the frequency of reports of similar inclusions not only in the salivary glands and kidneys but also in other parts of the body such as the lungs, liver, thyroid and so on. But Kuttner and Wang[19] say that an analysis of the cases shows "that in those instances in which the submaxillary and parotid glands are reported as involved, inclusions are not found in the other tissues of the body." This line of reasoning has little force unless it is clear that all the organs concerned have been equally thoroughly examined and that negative observations are reported as regularly as positive ones. Rats may exhibit both salivary gland and renal inclusions but it is not known whether there is a coexistence of the two in single individual rats or not. In other species restriction to one locality or the other is definite and impressive:

Guinea pigs (many investigators) — salivary glands, not kidneys.
14 moles [5] — salivary glands, not kidneys.
2 *Cebus fatuellus* [4] — salivary glands, not kidneys.
16 *Macacus rhesus* — kidneys, not salivary glands.

The nuclear inclusions, whether in what we call the latent state characterized by uniformity and rarity in untreated *Macacus rhesus*, or in the active condition suggested by abundance and diversity after the administration of irradiated ergosterol, indicate the possibility of a virus being present in the kidneys without any attention

being called to it by any clinically recognizable symptoms of disease. From the difference in the location of the affected cells and in the appearance of the inclusions it is probable that the hypothetical virus differs from the other hypothetical virus responsible for the formation of the inclusions described by Stewart and Rhoads [17] and by Covell,[18] so that two inapparent viruses may exist in the *Macacus rhesus*, the monkey most frequently used for experimental purposes.

Summary

Intranuclear inclusions suggestive of virus action were found in the kidneys of 12 out of 16 *Macacus rhesus* given repeated doses of irradiated ergosterol, of 1 out of 10 normal controls, and of 18 out of 107 pathological controls consisting of animals employed in the laboratory in a variety of experiments but not given irradiated ergosterol.

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DESCRIPTION OF PLATES

PLATE 90

Photomicrographs at a magnification of 1600 diameters of hematoxylin and eosin-stained sections of renal tubules of Monkey 89 treated with irradiated ergosterol, as detailed in the text.

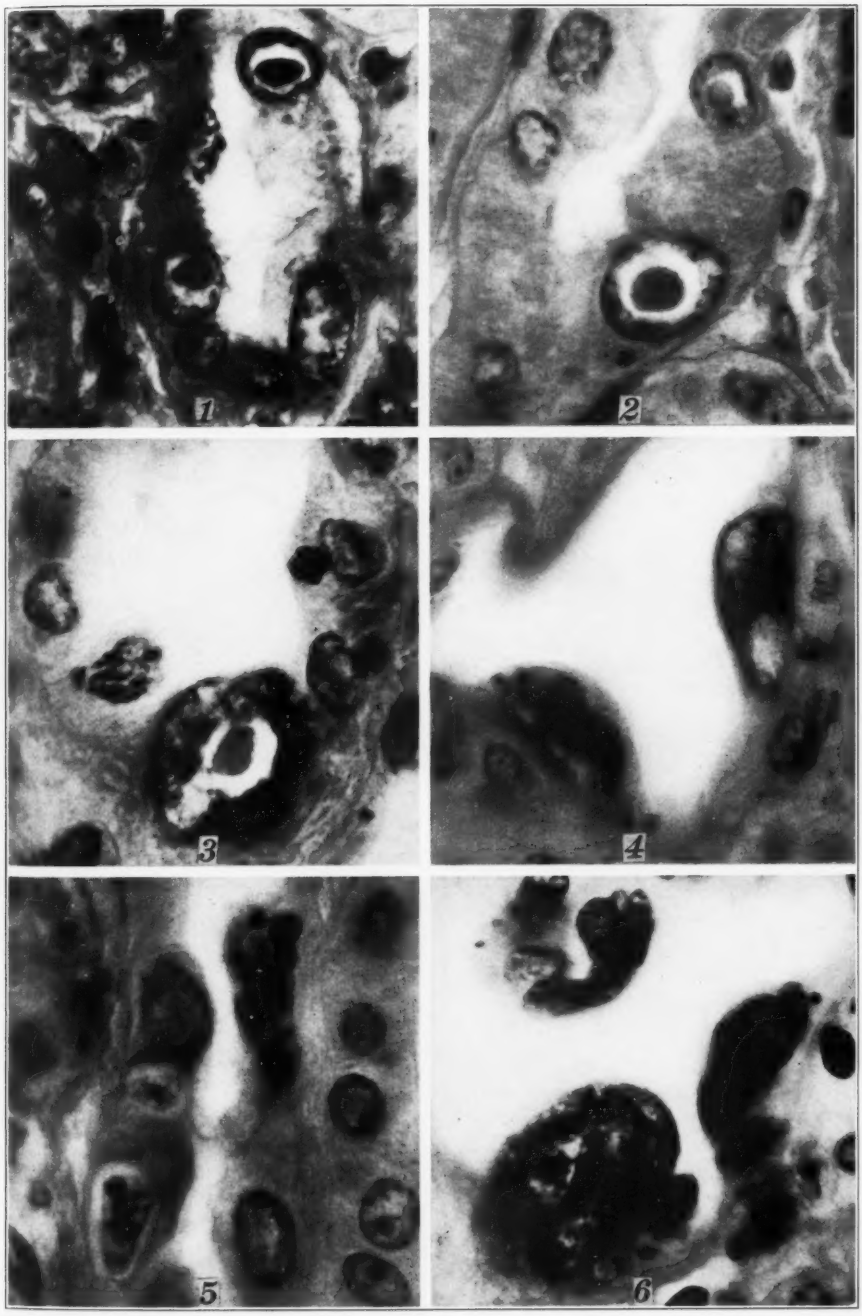
FIGS. 1 and 2. Acidophilic inclusions of variable size surrounded by clear halos in nuclei, some of which are enlarged while others are not.

FIG. 3. A greatly hypertrophied nucleus in the lower central part of the figure which contains an inclusion.

FIG. 4. Two nuclei, hypertrophied, slightly flattened and vacuolated but without distinctive inclusions.

FIG. 5. In the upper right hand side of the tubule is a nucleus that has undergone extensive budding.

FIG. 6. Extreme degree of enlargement and polymorphism of nuclei without the formation of inclusions.



Cowdry and Scott Nuclear Inclusions in Kidneys of *Macacus Rhesus*

PLATE 91

Photomicrographs at a magnification of 1600 diameters of nuclear inclusion-laden cells in monkeys that were not given irradiated ergosterol.

FIG. 7. Monkey 111, experimental poliomyelitis. Single, large, spherical inclusion-containing cell.

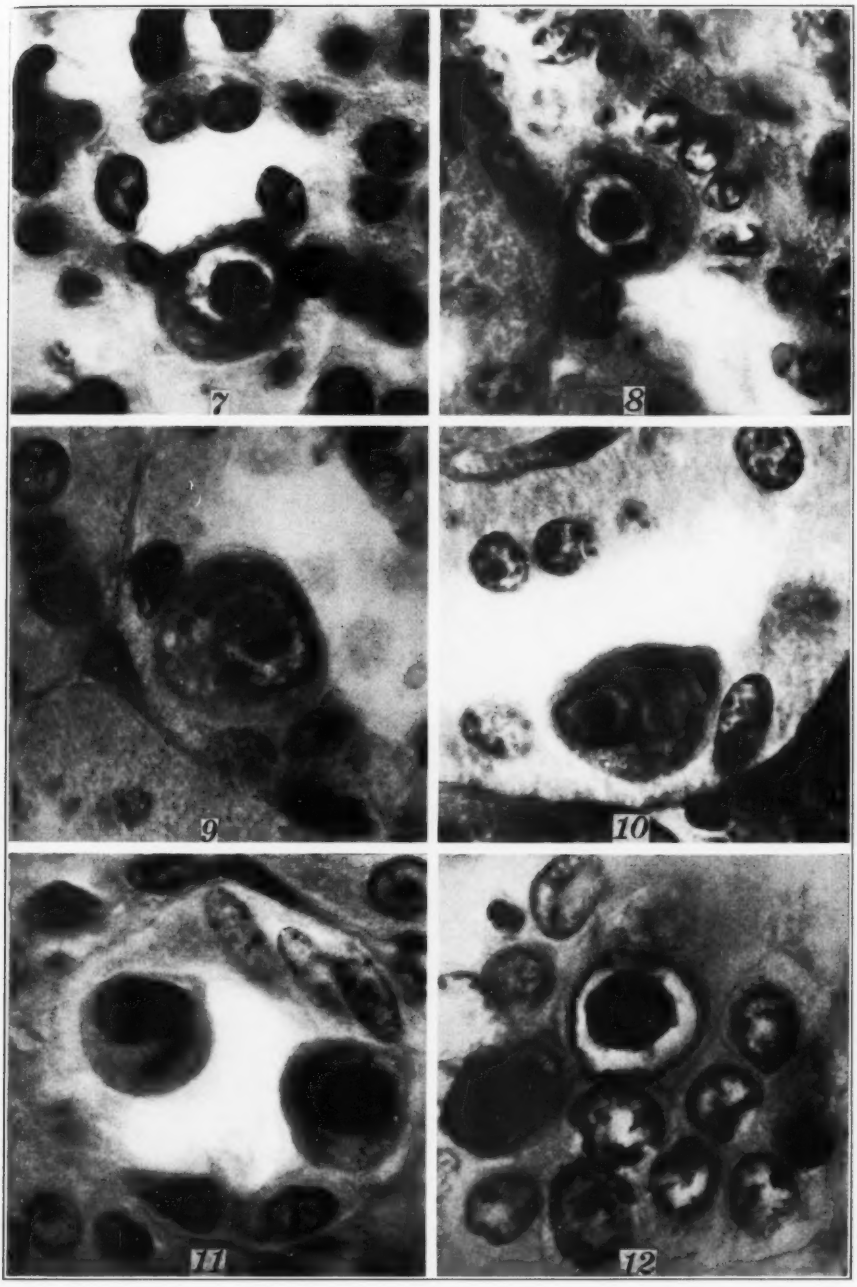
FIG. 8. Same kidney. A similar cell free in the lumen or attached to the wall at a higher or lower level in the tissue.

FIG. 9. Monkey 57, experimental poliomyelitis. Margination of the nucleolus on the nuclear membrane is evident. Basophilic cytoplasmic inclusions.

FIG. 10. Monkey 62, experimental poliomyelitis. A binucleated cell. The nucleus to the left contains a typical inclusion. The nucleus to the right is not shown because it was on a different focus. The cytoplasm was quite acidophilic.

FIG. 11. Monkey 40, cause of death unknown. Two inclusion-laden cells.

FIG. 12. Monkey 172, cause of death unknown. Epithelium of duct of Bellini cut at an acute angle with the surface. Two nuclei with inclusions.



Cowdry and Scott Nuclear Inclusions in Kidneys of *Macacus Rhesus*

NEUROPATHOLOGY OF EXPERIMENTAL VITAMIN DEFICIENCY *

A REPORT OF FOUR SERIES OF DOGS MAINTAINED ON DIETS DEFICIENT IN THE B VITAMINS

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INTRODUCTION

Recently a number of workers have tried to produce lesions of the nervous system in animals with diets deficient only in the vitamin B complex or in one portion of it. The divergent results of these observations [1, 2, 3] may be due to several causes. Diets made up of natural foods vary in composition in a manner that is at present little understood; thus, only diets made up of artificial foods can be relied upon to produce the desired vitamin deficiency. Furthermore, different species of animals vary greatly in their capacity to live on a deficient diet without manifesting symptoms. The rat, for example, if development of polyneuritis is used as a criterion, has been shown to be quite resistant to the effects of a diet deficient in vitamin B. Even animals of the same species vary in their susceptibility to deficient diets. The type of food on which the animal has been living before the beginning of the experimental regimen may account to some extent for these individual differences. Finally, the length of time that an animal lives on the deficient diet is a consideration that has not been sufficiently stressed. When an animal is completely deprived of vitamin B, it loses its appetite and may die rapidly of starvation. Under these conditions, Woollard [4] and, later, Kon and Drummond,[5] have concluded that the minor nervous system lesions which they have been able to discover can be reproduced by inanition and cachexia alone. Their animals, however, appear to have

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been deprived of vitamin A at the same time that they were starved, and this may account for the lesions.[6]

Cowgill [7, 8] has done extensive work in the development of artificial diets, and has produced severe nervous symptoms in dogs fed on an artificial diet almost entirely deficient in the vitamin B complex, but presumably adequate in every other respect. Goldberger and others,[9] working with dogs, have been able to show that dried yeast contains two components, one of which is heat labile, the antineuritic factor, vitamin B_1 or F, and the other heat stable, which they considered the pellagra-preventing factor and which is now known as vitamin B_2 or G. Stern and Findlay [10] studied two series of rats fed on diets deficient in vitamins B_1 and B_2, respectively. They found early degeneration of the myelin in the peripheral nerves and chromatolytic changes in the ganglion cells of the spinal cord when vitamin B_1 was lacking, and only vacuolization with lipochrome deposits in the ventral horn cells of the spinal cord when there was a deficiency of vitamin B_2. Because of the resistance of rats, it is surprising that these authors were able to find so much evidence of pathological changes in their animals.

Gildea, Kattwinkel and Castle,[11] using a diet described by Cowgill [7] as deficient in the vitamin B complex, were able to reproduce the nervous system symptoms in dogs which Cowgill had reported as due to polyneuritis. They emphasized the fact that the symptoms suggested disturbance mainly of the central, rather than of the peripheral nervous system. In their first series of dogs maintained on the Cowgill diet, deficient in both vitamins B_1 and B_2, the affected animals were repeatedly treated with vitamin B concentrates * until they improved, and then were allowed to develop symptoms again. After a number of such therapeutic attempts the process became irreversible and large doses of the concentrate no longer brought about a recovery. Grinker,[12] working with rats, has questioned the severity of these symptoms, and the existence of a definite spastic paralysis. Cowgill [7] published, in 1921, pictures of dogs that had developed "paralysis" while subsisting on a diet of the same type as the one employed in these experiments. In Figure 1, pictures taken from a motion picture film of the dogs studied by Gildea and his associates are presented as a final answer to Grinker's

* Yeast vitamin B (Harris Laboratories); alcoholic extract of wheat embryo (courtesy of Eli Lilly and Company). See appendix.

criticism. These illustrations, which represent the condition of the animals when therapy with vitamin B concentrates was no longer able entirely to clear up the symptoms, are necessarily inferior to the projected film, but they show spasticity of the legs, with marked disturbances of equilibrium upon walking or running. Even in the last stages of the condition, when in some animals there was complete loss of motor power of the hind legs, the knee jerks remained active, as shown in Figure 1*C*.

Gildea, Kattwinkel and Castle [11] reported that sections of the nervous systems of their dogs (Figs. 2, 3, 4 and 5) revealed evidence of myelin degeneration in the spinal cords, and in 3 out of 8 animals in the peripheral nerves. These lesions were first observed in the Weigert-Pal preparations. In order to confirm the findings, additional sections of the cords were stained with Spielmeyer's technique and corroboration of the lesions in the Weigert-stained sections was apparently obtained. Shortly thereafter Zimmerman and Burack [1] reported a study of the nervous systems of dogs that had rapidly developed symptoms on a similar Cowgill diet which was thought to be deficient in vitamins B_1 and B_2. They found demyelinization of the peripheral nerves, but were unable to find lesions in the spinal cord. Moreover, they observed some evidence of degeneration in the nerves of their control animals. They pointed out that the lesions in the Spielmeyer preparations of Gildea and his associates were probably artefacts. We have found Zimmerman to be correct in his criticism of some of the Spielmeyer-stained material, but we have been unable to discover any reason to question the lesions shown in Weigert-Pal preparations.

It is noteworthy that in their first series of experiments Zimmerman and Burack made no attempt to prolong the lives of the animals, or to create a state of chronic dietary deficiency. Recently they [13] have completed a study of the nervous system of 8 dogs which had been on a diet similar to the first one used by Gildea, Kattwinkel and Castle, except for the fact that they were given a B_1 concentrate (considered to be free of B_2) from the beginning of the experiment. In contrast to the first series of dogs studied by Zimmerman, these animals lived for as long as 300 days. In the 2 dogs of the series that died first, no changes in the spinal cord were observed. In the 6 animals that lived longer, the pathological changes consisted in a marked demyelinization of the peripheral nerves,

degeneration of the medullary sheaths, and replacement by gliosis of the dorsal columns of the spinal cord, particularly the fasciculi graciles. Degeneration of the medullary sheaths of the dorsal and often of the ventral nerve roots of the cord was found, and occasionally there were slight degenerative changes in many of the other fiber tracts of the cord. In contrast to their former observations it would appear that in this last series Zimmerman and Burack have reproduced in a thorough manner the *chronic* deprivation of vitamin B which was sought in our early experiments, and so have obtained corresponding pathological changes. Their observations, however, indicate that deficiency in the vitamin B_2 component may be responsible for the development of the lesions of the nervous system. In the light of our experiments and of the results of Zimmerman and Burack, the failure of Grinker and Kandel [3] to find evidence of lesions of the nervous system in rats deprived of the vitamin B complex seems probably to have been due either to the fact that these animals died in acute illness, or to the unsuitability of the species for this study.

Original Observations

Since 1928 we have made four attempts to throw light on the relation of vitamin B to lesions of the nervous system, especially of the spinal cord. The particular objective was to discover whether deficiency of vitamin B had a relation to those types of "combined system disease" encountered in pernicious anemia, in pellagra, and in chronic alcoholism. Thus, the first problem was to find out whether a disturbance of the central nervous system could be produced by deficiency of vitamin B, and whether the clinical symptoms of this syndrome could be accounted for by demonstrable pathological lesions. The present report confirms the fact that this objective was attained despite the criticisms of Zimmerman and of Grinker. The second problem was to discover whether vitamin B_1 was the deficiency involved, and whether inanition played a part in the production of the symptoms and lesions. Lastly, since the clinical condition was to a certain extent reversible, an attempt was made to determine whether or not the pathological lesions could be diminished by the use of a therapeutic agent. Observations were made throughout on the relation which peripheral neuritis bore symptomatically and pathologically to the syndrome.

Series I

Series I consisted of 8 dogs that were put on a diet devised by Cowgill [7] (Diet I)* and supposed to be deficient in the vitamin B complex. After approximately a month of this diet all of the dogs developed anorexia, listlessness or weakness. An effort was made to maintain a chronic condition in these dogs by giving them extract of wheat embryo or yeast when marked neurological signs, often including convulsions and coma, had appeared. Except when the disturbance was too far advanced, they responded within 24 hours with an initial stage of marked general improvement. Then followed a slower second stage of recovery lasting several days, during which residual spasticity or ataxia of the hind legs was gradually relieved, but with progressive difficulty in subsequent relapses. Thus in successive relapses, after the initial relief of convulsions and coma, the hind legs especially were stiff and weak, and in some animals finally became paralyzed. Although it is difficult to be certain of sensory symptoms in animals, the behavior of these dogs, their awkwardness, and the misplacement of their limbs certainly suggested that the perception of deep sensibility was abnormal (Fig. 1). In general their reflexes were normal or hyperactive, and they appeared to have neither loss of skin sensation nor tenderness over the nerve trunks. Convulsions occurred in all but Dog 1. Tetany and opisthotonos were observed. Death finally occurred following convulsions in Dogs 2, 3, 4, 5 and 7. Dogs 1, 6 and 8 died quietly. The animals lived from 2 to 8 months.

Autopsies were done as soon after death as possible. Grossly the findings were not remarkable. The alimentary mucosa was usually injected and occasionally there were minute hemorrhages. The brain and cord were usually somewhat hyperemic. Histological study of the central nervous system showed no definite cortical or cerebellar lesions in sections stained by the Weigert method, although in most cases Nissl stains showed that the cerebral nerve cells and the Purkinje cells were degenerating. Fat was present in varying amounts in the nerve cells and perivascular spaces in the cerebral cortices of all animals. The cords showed definite myelin lesions (Weigert) in Dogs 1, 2, 3, 5 and 6 and less clear-cut lesions in Dogs 7 and 8 (Figs. 2, 3, 4 and 5). The ventral horn cells were in

* For diets, see appendix.

poor condition (Nissl), and in some instances exhibited satellitosis. Fat was present in small amounts in the nerve cells of the spinal cords of Dogs 1, 2, 5, 6 and 7. The amount of fat in all of these sections was probably not significantly greater than in normal controls. Peripheral neuritis was considered present if more than 10 per cent of the fibers in a nerve trunk contained material stainable with scharlach R or with osmic acid by the Marchi technique. By this criterion Dogs 1, 3 and 8 had peripheral neuritis.

Series II

The second series of 6 dogs was kept on the same Cowgill diet, with the addition of autoclaved yeast (Diet II). It was thought that this diet was deficient only in vitamin B_1, the antineuritic vitamin. Within a month, as with Series I, the dogs showed loss of appetite, weakness and apathy. They did not seem to respond to vitamin treatment as well as the animals of Series I. This may have been due to the fact that not so much time was spent in tube feeding and other efforts to prolong life. In general they had progressive weakness and ataxia, followed by opisthotonos and convulsions with or without tetany. No paralysis was observed. The reflexes were always present, usually hyperactive, and the dogs exhibited no flaccidity or tenderness over the nerve trunks. They lived a much shorter time (an average of 2 to 3 months less) than the animals in Series I. All of them were dead within 6 to 14 weeks after the beginning of the experiment.

At autopsy the organs appeared to be normal in gross, except for the injection of vessels in the alimentary mucosa and in the brain and cord. Dogs 2 and 6 had opacity and erosion of the cornea. Dog 6 had an apparent increase in cerebrospinal fluid. Histological study of the central nervous system showed a questionable myelin lesion (Weigert) in the cortex of Dog 1, which was corroborated by a clearly positive fat stain. A definite lesion (Weigert) occurred in the cerebellum of Dog 6, as well as a lesion of the pyramidal tract in the medulla. Nerve cells in the cortex and cerebellum were found to be undergoing degenerative changes, but to a lesser degree than in Series I. Fat was present (scharlach R stain) in small amounts in the cortices of Dogs 1, 3 and 6, and in the cerebella of Dogs 2, 3 and 6. Definite lesions (Weigert) occurred in the cord of only 1 ani-

mal, Dog 6, which lived approximately 3 months (Fig. 6). The cresyl violet-stained section from the same cord showed gliosis. Practically no fat was found. Peripheral neuritis occurred in Dogs 2 and 6. In short, only 2 of the 6 animals had significant central nervous system lesions.

Series III

Series III was designed to determine what relation inanition bore to the clinical and pathological conditions observed in Series I and II. Seven dogs (1 to 7) were placed on the original Cowgill diet, deficient in the vitamin B complex (Diet I). Three additional animals (2A, 3A and 4A) were given daily amounts of a similar diet with the addition of 4 per cent by weight of granulated unheated yeast (Diet II) equal in weight to the amount of the vitamin B-deficient food eaten the previous day by Dogs 2, 3 and 4, respectively. Dogs 4 and 4A were each given also 8 cc. of cod liver oil daily. No attempt was made to prolong life by treatment with vitamin B concentrates.

With the exception of Dog 3, all 7 of the animals that were on diets deficient in the vitamin B complex (Diet I) showed the symptoms described before — anorexia, spasticity and convulsions. In general they showed less ataxia than did the dogs of Series I and II, and their symptoms were less severe. They lived from 32 days to 4 months, and all but Dogs 1 and 5, which were found dead, were killed with chloroform. Dog 5 had had very severe symptoms and lived $3\frac{1}{2}$ months. Dog 3 showed no symptoms except mild loss of appetite. It was killed after $3\frac{1}{2}$ months, together with its control, Dog 3A. Because Dog 3 had not had much anorexia, Dog 3A had not had much inanition. Nevertheless, Dog 3A had been spastic, with exaggerated reflexes, for about a week before it was killed.

The autopsies were essentially negative in gross. Dogs 1 and 4 had corneal opacity and a purulent conjunctival discharge. Pneumonia was found in Dogs 2 and 4. In Dog 1 there was blood in the stomach and intestines. Histopathological changes in the cord were entirely absent except in Dog 5. Since this dog was found dead, and in rigor, the myelin change was probably the result of postmortem autolysis. Dogs 2, 3 and 4 showed definite peripheral neuritis, which is interesting in view of the fact that reflexes were absent in Dog 2. None of the animals showed tenderness over the nerve trunks.

Series IV

Series IV consisted of 10 dogs kept on the original Cowgill diet (Diet I) deficient presumably in the entire vitamin B complex. The experiment was designed to determine to what extent the pathological process was reversible. We hoped to demonstrate lesions in the animals that became sick and were allowed to die untreated, and either smaller lesions or none at all in the animals that were treated with tiki-tiki * during the acute phases of the illness, and with yeast (Diet II) every day during the following period. These dogs, unlike those of Series I, were not allowed to relapse repeatedly, but were treated as soon as convulsions appeared and continuously thereafter.

Four of the animals, Dogs 2, 3, 4 and 10, developed symptoms within 54 to 95 days after the beginning of the experiment. The symptoms, as before, were anorexia, spasticity, ataxia, paralysis, and finally convulsions. Therapy consisted in giving from 3 to 4 cc. of tiki-tiki in each case and in supplementing the diet from then on with 1 gm. of granulated yeast daily per pound of dog. The 4 dogs lived on this diet from 69 to 105 days, and all finally showed complete clinical recovery. They were killed with chloroform. The untreated animals, Dogs 1, 5, 7 and 8 became ill within 58 to 88 days and, with the exception of Dog 5, showed all the symptoms listed above. Dog 5 was extremely spastic after 67 days, but died without having convulsions. Dogs 7 and 8 were chloroformed. Dogs 6 and 9 showed no symptoms except a slight amount of anorexia.

The autopsies, as usual, were not remarkable upon gross inspection. Histological study showed no myelin change in any cord that we could not duplicate in our series of controls. Poliomyelopathy, consisting in disintegration of nerve cells, or in satellitosis with degenerative changes in most of the nerve cells, occurred in all of the dogs to a significantly greater degree than was found in our series of control animals. We could not demonstrate any less poliomyelopathy in the cured animals or in the symptomless dogs than in the dogs that died in acute illness. Fat stains were negative throughout. Peripheral neuritis was present only in Dog 4, and was minimal.

* See appendix.

Discussion

Of the four groups of animals, Series I, given a diet deficient in the entire vitamin B complex, was the only group in which definitive myelin lesions were consistently found in the spinal cords. These dogs were the only ones in which repeated treatments with vitamin B concentrates were used to prolong life. Since in Series III and IV an exactly similar diet was given to 17 animals, but with no particular attempt to create a chronic deficiency, so that the animals died sooner, it is reasonable to conclude that a prolonged dietary deficiency is necessary in order to produce demyelinization and other lesions of the central nervous system in dogs. This fact very possibly explains the failure of Grinker and Zimmerman to confirm our original observations. As mentioned above, however, Zimmerman has recently found marked lesions in dogs which were fed with great care and persistence until they finally died at the end of about 300 days. These animals were given a similar diet but with the addition of a vitamin B_1 concentrate from the beginning of the experiment. Deficiency of vitamin B_1 is thus apparently not the basis of the morphological lesions.

In Series II deficiency of vitamin B_1 was found to produce severe disturbances, with a clinical picture including convulsions and coma. As in Series I, and as had previously been observed by Cowgill,[7] the administration of vitamin B concentrates had a rapidly beneficial effect. This result is in agreement with the belief of Findlay,[14] Kinnersley and Peters,[15] and Gavrilescu and Peters [16, 17] that the syndrome produced by vitamin B_1 deficiency must, in part at least, be accounted for by a "functional" disturbance of the central nervous system, *i.e.* by one not demonstrable by our *present-day histological methods*.

In Series I and II we were not able to demonstrate lesions (Weigert) in the cortex, although in 1 animal (Dog 6, Series II) we found a definitive myelin change (Weigert) in the cerebellum. The presence of fat in the cerebral cortices, both in nerve cells and in scavenger spaces, of all the dogs of Series I and in 3 of the dogs of Series II leads us to believe that there were definite lesions present. The condition was probably not sufficiently advanced to produce myelin lesions. Cord lesions, which occurred in similar areas in a number of sections, were demonstrated by the Weigert-Pal technique in 7 of

the 8 dogs of Series I but in only 1 of the 6 dogs of Series II (Figs. 2, 3, 4, 5 and 6). It was impossible to check these lesions with any other stain. The fat-stained sections were invariably negative, and the Nissl-stained sections showed only minimal changes. Mucicarmine stains were done on both series and showed nothing abnormal. Marchi stains unfortunately were not done.

In Series III and IV, despite acute symptoms, little or no morphological evidence of myelin lesions was observed. Because of the symptoms of Dogs 3 and 3A (Series III) we conclude that either inanition may produce a small part of the clinical syndrome (spasticity), or else a diet that has an adequate vitamin content for most animals may be deficient for certain individuals. In Dogs 2, 3 and 4 of this series histological peripheral neuritis is shown to bear an entirely inconstant relation to the clinical symptoms, having been found in the 3 animals that exhibited, respectively, flaccidity, no symptoms and spasticity.

Conclusions

1. Seventeen dogs given a diet deficient in vitamin B (Cowgill) developed signs of acute disturbance of the central nervous system and died without treatment with vitamin B concentrates. Only minimal histological changes were found in the central nervous system.

2. Eight dogs given a similar diet, but whose acute neurological signs were repeatedly and temporarily relieved with vitamin B concentrates, developed gradually a residual degree of spastic ataxia and eventually motor paralysis, with reflexes present. Definitive histological lesions of the central nervous system were found in all but 1 animal.

3. Nissl stains of the cerebral and Purkinje cells and of the ventral horn cells revealed evidence of degeneration. Weigert-Pal stains of the spinal cords showed definite losses of myelin in 7 dogs. The peripheral nerves of 3 dogs showed an increase of material staining with scharlach R or with the Marchi technique.

4. The results of observations on the effect of partial starvation, of supplements of cod liver oil, and of therapy with dried yeast on morphological changes in the central nervous system were rendered inconclusive, probably because the basic deficiency was not sufficiently prolonged to produce morphological changes in the nervous system of any of the animals in such experiments.

APPENDIX

Diet I [7]

20 gm. of this diet were given per kilo of body weight

	gm.
Commercial casein water-washed grade	6.3
Cane sugar	4.5
Butter fat	1.1
Lard	2.8
Bone ash	0.4
Salt mixture, Karr [18] *	0.2
Water	4.7

* Sodium chloride, 10 gm.; calcium lactate, 4 gm.; magnesium citrate, 4 gm.; iron citrate, 1 gm.; Lugol's solution, few drops.

Diet II

Basal diet as above, with the addition of 4 per cent by weight of autoclaved Fleischmann's yeast

. . . . . .

Washed and purified casein from A. H. Thomas Company, and Adler Company, Philadelphia, Pa.
Dried yeast, Fleischmann Yeast Company, Boston, Mass.
Bone ash, Howe and French, Boston, Mass.
Yeast vitamin B extract, Harris Laboratories, Tuckahoe, N. Y.
Alcoholic extract of wheat embryo, Eli Lilly and Company, Indianapolis, Ind., supplied by Dr. G. H. A. Clowes.
Tiki-tiki, alcoholic extract of rice polishings, Bureau of Science, Manila, P. I.

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DESCRIPTION OF PLATES

PLATE 92

FIG. 1. Single exposures taken at short intervals from a motion picture film of Dogs 2, 5 and 6 of Series I. The pictures demonstrate the spasticity of the legs, with marked loss of equilibrium on walking or running, and the retention of the knee jerk with complete motor paralysis in the final stages of the condition.

(*A*) Dog 5 with wide base of hind legs and spasticity. Note that the dog is jumping clear of the ground in the second picture, and after landing on rigidly extended legs attempts to run, but falls over in a rigid attitude.

(*B*) Dog 5 demonstrates particularly well the spasticity of the hind legs, which are shown lifted completely off the ground in the third picture, before falling.

(*C*) Dog 6 in the final stage, with complete motor paralysis of the hind legs. Note, however, that the knee jerk is present, causing blur in the fourth picture.

A *B* *C*

PLATE 93

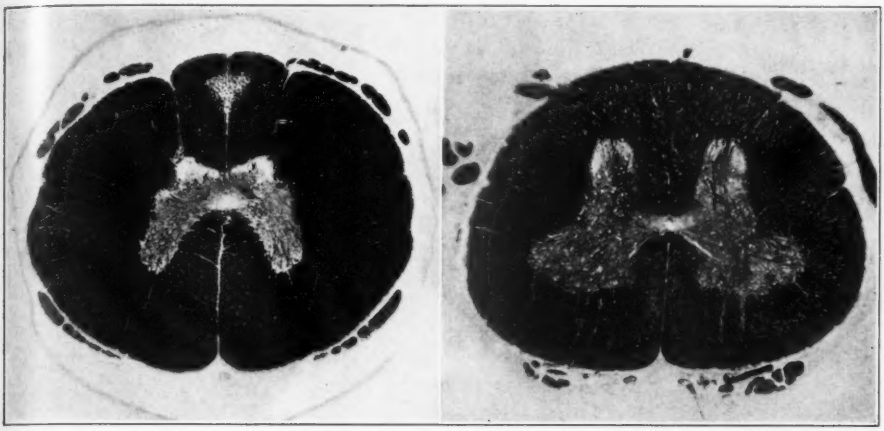
FIGS. 2, 3, 4 and 5. Photomicrographs of sections from the cords of 4 dogs of Series I which died after 4 to 6 months on a diet deficient in the vitamin B complex. Weigert-Pal stain. × 10.

FIG. 2 (Dog I, Series I). Shows a symmetrical, circumscribed loss of myelin in the dorsal columns.

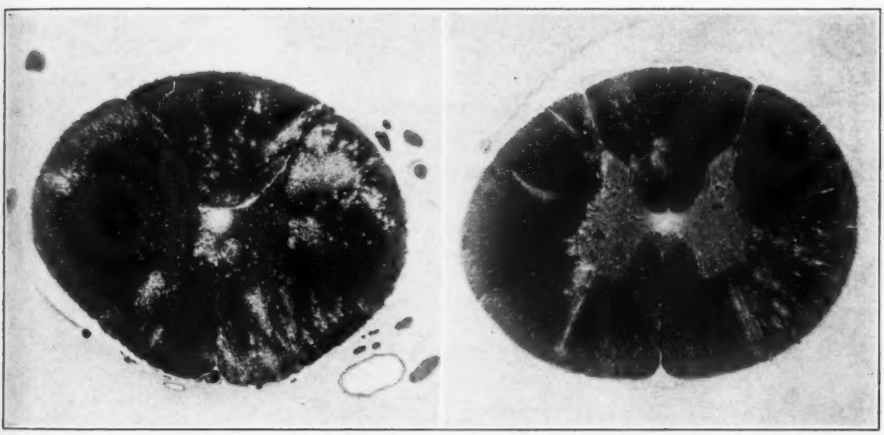
FIG. 3 (Dog 7, Series I). Shows a more diffuse but definite loss of myelin without symmetrical distribution except in the uncrossed pyramidal tracts along the anterior fissure.

FIG. 4 (Dog 6, Series I); and FIG. 5 (Dog 2, Series I). Show definite but irregularly distributed loss of myelin.

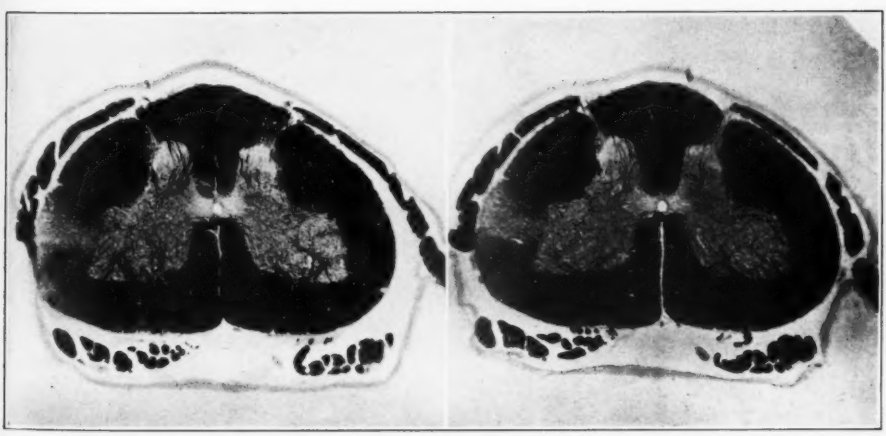
FIG. 6. Photomicrographs of two sections from the same block of the cord of Dog 6, Series II. This dog had lived for 3 months on a diet deficient in vitamin B_1 and died in convulsions. Both sections show a loss of myelin in the same area. The same lesion was observed in fourteen other sections from the same block. Weigert-Pal stain. × 10.



2 3



4 5



6 6

MONOCYTES AS A SOURCE OF ALVEOLAR PHAGOCYTES *

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Conclusions regarding the origin of the alveolar phagocytes of the lung have been controversial since the earliest studies. The more probable sources of supply, namely, the alveolar epithelium, the macrophages of the pulmonary connective tissue, the monocytes of the circulating blood and the capillary endothelium have all been extensively investigated and championed. It has been our purpose to bring fresh evidence to the problem in an endeavor to settle the question definitely if possible. Many ingenious methods have been utilized in attacking this question, yet none has been entirely successful. Perhaps the most pertinent factor that has made the proof of the issue so difficult is the fact that no method has been unassailably successful in marking the progenitor of the alveolar phagocyte in such a fashion that its progress from the source of supply to its position as a free cell in the alveolar spaces could definitely be followed. In passing from the circulation to the alveolar space a monocyte may become marginated, migrate through the capillary wall, travel through the interalveolar stroma and pass between the alveolar lining cells to gain the air sac. In fixed tissues the cell is found at that point in its journey where it was at the moment that fixation occurred. Such a cell might, because of its location, be mistaken for a capillary endothelial cell, a resting histiocyte of the stroma, a septal cell or an alveolar epithelial cell. We have, by the method to be described subsequently, eliminated this obstacle by obtaining marked viable monocytes from one animal, injecting them into the circulation of a second animal and observing the behavior and distribution of the transferred cells in the new host.

It is not necessary again to analyze the literature that has accumulated on the subject since this has been accomplished recently by both Foot [1] and Fried.[2] We must, however, present briefly the prin-

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cipal views of various investigators in order to provide a basis for the analysis of our own experiment. They have been summarized recently by Haythorn [3] in a paper on multinucleated giant cells and from that work we again quote the conclusions of leading investigators as they pertain to the issues of this problem.

The alveolar epithelium has been accepted as the origin of alveolar phagocytes by many, among whom are Sewell,[4] Gross,[5] and perhaps most recently, Cappell.[6] They have reported that cells lining the alveolar spaces are able to phagocytose various suspensoid dyes injected intratracheally and have shown how these cells, having ingested various dyes, may swell, desquamate and subsequently appear as free phagocytes in the alveolar spaces. It is an indisputable point that, following the intratracheal injection of irritant dyes, one may see in the alveolar spaces many phagocytic mononuclear cells that have ingested the injected pigment. The cells may be either free in the alveolar spaces or applied to the alveolar walls. However, the evidence that these phagocytes are epithelial cells is not convincing. We have duplicated such pictures in which the rôle of the epithelium was positively excluded. In our studies we have often seen phagocytic mononuclears that had escaped from the blood stream so closely applied to the alveolar walls that they could not be distinguished from alveolar epithelium.

Gardner and Smith [7] have studied paraffin sections of lungs vitally stained with neutral red and concluded that the phagocytes came not from endothelial or epithelial cells, but from interstitial septal cells of the alveolar walls, and that they should therefore be considered as connective tissue phagocytes. They washed free of blood the lungs which they used and concluded that they had eliminated the circulating monocytes as a source of supply. Yet it has not been proved that the cells which they saw in the interstitium had not been originally derived from the circulating mononuclears. Whence the resting histiocyte is derived is still in dispute and since Foot [1] has demonstrated that these histiocytes, together with circulating monocytes and alveolar phagocytes, have a mutual and specific affinity for certain silver dyes, we believe that one may merely represent the transitional form of another. Hence the phagocytes that appeared in their sections may have been ultimately derived from the circulating monocytes. In further support of this contention is the work of Cappell [8] who demonstrated that marked free mononuclears in sterile

peritoneal exudates became resting histiocytes when the irritation had been permitted to subside. Fried [2] recently also has advanced the belief that the pulmonary macrophage or histiocyte is the progenitor of the alveolar phagocyte. He injected various irritants intratracheally and observed an abundance of cells that had phagocytosed the injected material free in the alveolar spaces. His conclusions were that the observed phagocytes were derived from a macrophage system resident in the pulmonary parenchyma itself. He, however, accepts a mesodermal origin for the alveolar lining cells and thereby automatically eliminates alveolar epithelium as a source of supply. Even if this unproved point were definitely established he does not offer proof to exclude any of the leukocytes of the circulating blood as an equally potent source of supply.

Whether or not one accepts the capillary endothelium as a possible source of the alveolar phagocyte is dependent on whether or not one believes that capillary endothelium supplies the wandering mononuclear phagocytes. Mallory [9] was the first to propose this view and he has had many followers, among whom are Foot,[10,11,12] Permar,[13,14] Medlar,[15,16] McJunkin,[17,18] Haythorn,[3] and others. Although we believe that there is strong evidence in favor of this theory we have not attempted to add any positive evidence to it in this work. We have noted many times that pigment-laden mononuclears which were injected into the circulation have appeared in the pulmonary capillaries in such positions as to render them indistinguishable from the capillary endothelium, but when simple unphagocytosed suspensoids were introduced into the circulation we have never observed capillary endothelium, *in situ*, to take it up. Cappell [6] and others have long preceded us in this observation. However, that sort of evidence does not exclude the possibility that these same endothelial cells, when released from the capillary wall, may become active phagocytes.

To our knowledge evidence in support of the belief that circulating monocytes may become alveolar phagocytes is rather sparse. Lewis [19] has observed carbon-laden monocytes leaving the pulmonary capillaries to enter the alveoli of the lungs of living frogs. Foot,[1] employing his method of silver impregnation, has recently concluded that alveolar phagocytes have their origin in the circulating monocytes. We believed that if we could obtain marked viable monocytes and inject them into the circulation of a homologous animal we should

be able either to establish or eliminate them as the parent cells of alveolar phagocytes. This we have attempted to accomplish by the method about to be described. Similar methods have been employed by Seemann and Theodorowitsch,[20] and Borghi [21] in their studies on the effect of the injection of homologous leukocytes in animals. Our procedure may be outlined briefly in the following steps:

1. A suspension of leukocytes was obtained by the production of a chemical peritonitis.

2. The cells were marked by a method of phagocytosis of suspensoid dyes *in vivo*. The suspension of marked cells was then removed, cleared of free pigment, washed and standardized.

3. The viability of the cells thus obtained was established by phagocytic tests *in vitro* utilizing a contrasting pigment.

4. The standardized marked viable suspension was injected into homologous animals at various points of their circulation.

5. The recipient animals were killed at various time intervals and the distribution of the marked cells was noted.

Method

An elaboration of the technic outlined above is as follows:

1. *Production of the Exudate:* Adult guinea pigs were used as donor animals. They yielded an exudate rich in mononuclears by the use of a single intraperitoneal injection of 5 per cent aleuronat and 3 per cent starch in plain broth.

2. *Marking and Standardizing the Cells:* At the end of 12 hours suspensoid material for phagocytosis was injected into the peritoneal cavities of the animals in which the leukocytic response had been induced for the purpose of marking the leukocytes by phagocytosis *in vivo*. The material consisted of: (*a*) 5 cc. of 30 per cent India ink or 5 cc. of 5 per cent lithium carmine; (*b*) 5 cc. of 1.5 per cent sodium citrate solution; and (*c*) 5 cc. of 50 per cent fresh guinea pig serum. Carbon in the form of India ink was used in the majority of cases because of its inert nature. We felt that carmine was a less acceptable substance because of its complex protein nature which we felt might be harmful to the cells that ingested it. Further, the phagocytic response of cells to carbon was greater than to carmine, probably because the carbon was present in more finely divided particles. The sodium citrate was merely used as an anticoagulant.

The fresh guinea pig serum was used because we found that its complemental action definitely enhanced phagocytosis, as can readily be seen by the data in Tables I and II. Experiments were carried out at the suggestion of Dr. G. H. Robinson,[22] who had previously established this point in a series of experiments that involved phagocytic

TABLE I

Effect of Complement on Phagocytosis in Vivo *

Amount of complement injected 50% guinea pig serum †	Carbon-containing cells	Estimate of quantity of pigment per cell
cc.	*per cent*	
none	20.5	+
2	28.2	+
4	40.0	++
8	43.4	++
4 (inactivated)	26.6	+
8 (inactivated)	28.4	+

* Number of cells counted in each instance 200.
† Diluted with normal saline

TABLE II

Effect of Complement on Phagocytosis in Vitro

Formula	Phagocytosis observed in	Carmine-containing cells after 30 min.	Amount pigment per cell
equal parts	*min.*	*per cent*	
Cell suspension, 2% carmine, normal saline ...	15	48	++
Cell suspension, 2% carmine, 50% complement	5	73	+++
Cell suspension, 2% carmine, 50% inactivated serum.....	12	56	++

tests. Our experiments were carried out utilizing both *in vitro* and *in vivo* measures. In both series we observed, as did he, that the addition of fresh complement enhanced phagocytosis with relation to three important points: (1) the increase in the number of pigment-containing cells; (2) the increase in the number of pigment particles per cell; and (3) the rapidity with which phagocytosis occurred. Fenn [23] has demonstrated that complement increases the phagocytic power of cells for inorganic substances. He explained this phenomenon on the basis of change in surface tension. We, however, believe that it is due to a stimulating action of complement on

the cells because, as is shown by the tables, when the complement is destroyed the addition of serum increases phagocytosis but slightly. Phagocytosis was permitted to progress in the peritoneal cavities of the donor animals for 12 hours. At the end of this time they were killed and the cellular exudate was removed by sterile pipettes after the abdominal cavities had been opened. Having obtained a suspension of marked cells we were still beset by the problem of clearing it of free pigment particles, inasmuch as it was essential not to inject any free pigment into the recipient animals. Curiously enough, it was found that simple passage through a paper filter yielded a filtrate which contained no free dye particles. The filtration was carried out in a dark incubator at 37° C. Fenn [23] has shown that this is the optimum temperature for maintaining the viability of cells and Earle [24] has demonstrated the destructive action of light on such suspensions. Why dye particles, infinitely smaller than cells, were retained on the filter, whereas the cells passed into the filtrate, can probably be explained by the theory of electrical charge in filtration, as advanced by Mudd [25] and others. As applied to our observations, the dye particles possessed a charge opposite to that of the filter and therefore adhered to it, whereas the cells possessed a like charge and passed through the pores without difficulty.

In order to make injection of the suspension practical it had to be concentrated to small bulk. Centrifugation did not damage the cells appreciably when carried out at low speed with the balancing cups containing water warmed to 40° C. The supernatant fluid was poured off and the cells were suspended in 50 per cent guinea pig serum. Standardization consisted of counting the cells of such a concentrate and diluting it so that each cubic millimeter contained approximately 50,000 cells. By making differential smears it was found that more than 80 per cent of the pigment-bearing cells were phagocytic mononuclears.

3. Method of Establishing Cell Viability: This was accomplished by observing whether the pigment-bearing cells would phagocytose a contrasting pigment or not. The tests were carried out *in vitro* in an attempt to establish a state as closely simulating body conditions as possible. Therefore, hanging drop preparations suspended in guinea pig serum were observed on a warm stage. Agitation of such a preparation, it was found, increased the rapidity of phagocytosis. We felt, therefore, that the factor of speed of circulation could be

neglected. Hanging drop preparations were made using, for example, a loopful of carbon-containing cells, a loopful of 2 per cent carmine, the contrasting pigment suspension, and two loopfuls of guinea pig serum. Observations were directed toward four major points: (1) the percentage of all cells which phagocytosed carmine particles; (2) the ability of the carbon-containing cells to take up the contrasting pigment; (3) the length of time which elapsed before general phagocytosis began; and (4) the degree of phagocytosis at the end of 30 minutes. Analysis of a series of such preparations yielded the following averages. Phagocytosis was observed within 5 minutes and 73 per cent of all cells were seen to contain carmine particles at the end of 30 minutes. The majority of cells ingested so much carmine that their structure was obscured. Those mononuclear cells that were carbon-containing were equally as active as those that were free of carbon in respect to their avidity for carmine. This established the fact that over 50 per cent of the marked cells to be injected were viable. It is reasonable to assume that this percentage would be considerably higher under absolute body conditions.

4. *Method of Injection:* To rule out anthracosis several series of recipient animals used consisted of young guinea pigs raised outside of the city. Control sections of the lungs of similarly reared animals of the same age showed only widely separated particles of carbon. In one series the cell suspension was introduced into the jugular vein, in another directly into the heart, and in a third series into the portal vein. A control group received a pure suspension of India ink in normal saline solution. In regard to this group we found, as have many others, that suspensoid dyes such as India ink when injected intravenously possess no powers of diffusion. They are stored within the cells of the so-called reticulo-endothelial system and are never found in the lining cells of the alveoli or in the alveolar spaces in any appreciable amount.

5. *Preparation of the Histological Specimens:* The animals were killed with chloroform at intervals varying from 3 hours to 7 days. Sections were fixed in formalin and Zenker's fluid. The stains employed were eosin-methylene blue, Wright's, and simple hematoxylin. Unstained sections were also prepared. The simple hematoxylin method gave a very delicate stain in which carbon-containing cells contrasted sharply with cells free of the pigment.

Results

The microscopic picture of all animals was almost identical regardless of the time permitted to elapse before they were killed or the site at which the suspension was injected.

The dye-containing cells were found almost entirely in the lung tissue. Some were seen within the pulmonary capillaries, some appeared in the lung stroma, but the vast majority were found to be either free in the alveolar spaces or applied to the alveolar walls, apparently lining the alveolar walls and thereby presenting the morphological picture of alveolar epithelium. In several instances pigment-bearing mononuclears were seen escaping from pulmonary capillaries, the bulk of their cytoplasm being adherent to the alveolar walls in the position of epithelium and the remainder of the cell still within the capillary. Had we not known their origin we would have interpreted many of these cells as swollen epithelial cells which had ingested dye granules. Still others, situated in the connective tissue, would probably have been considered to be histiocytes of the stroma.

The liver, spleen and bone marrow contained occasional dye particles. We are aware of the fact that some pigment-bearing cells were no longer viable when introduced into the circulation. Naturally they were disrupted with consequent liberation of their pigment. This free pigment was then taken up by the phagocytic system of the liver, spleen and bone marrow.

Discussion

We found that viable phagocytic mononuclears marked by the ingestion of pigment, when injected into the circulation of a guinea pig, were concentrated in the lungs. There they were identified as alveolar phagocytes, although those in the intermediary locations between capillary and alveolar space may well have been identified as capillary endothelium, macrophages or septal cells, had we not known them to be carbon-laden mononuclears which we injected into the circulation. The suspected progenitor, namely the phagocytic mononuclear, was found to appear as the alveolar phagocyte.

It is impossible to state, at present, the reason for the concentration of these cells in the lung. We can say, however, that it was not

due to simple capillary filtration for those cells injected into the portal vein were not filtered out in the liver but appeared in the lungs. Furthermore, marked cells were identified in differential smears from the circulating blood as much as 2 hours after their injection.

It occurred to us that perhaps the cells which we injected were destroyed in the blood stream of the recipient animals; that the liberated pigment was then phagocytosed by certain cells residing in the pulmonary parenchyma of the recipient. But this is impossible because suspensoids have no power of diffusion. When a solution of India ink was injected we were unable to discover any of it in the alveolar spaces or in cells lining their walls.

We have not attempted to establish monocytes as the sole origin of alveolar phagocytes under all conditions. Cunningham, Sabin and Doan,[26] and others grant monocytes a histiocytic origin. If this relationship is definitely established the problem of the alveolar phagocytes will be solved indisputably.

Summary and Conclusions

1. A technic for preparing a marked cell suspension in one animal and transferring it to other animals is described.

2. The complemental power of serum enhances the phagocytic properties of homologous leukocytes *in vivo* and *in vitro* by increasing the number of pigment-containing cells, the number of particles per cell and the rapidity of the process.

3. Viable phagocytic mononuclears marked by the ingestion of pigment are concentrated in the lungs regardless of the site of injection of the cell suspension.

4. Alveolar phagocytes are derived, largely if not entirely, from monocytes of the circulating blood.

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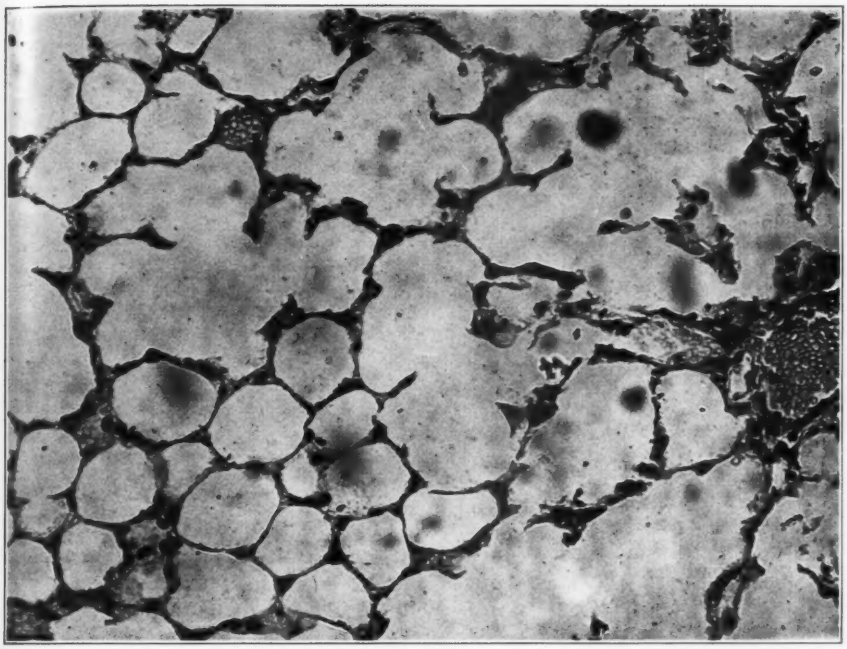
DESCRIPTION OF PLATES

PLATE 94

FIG. 1. A control lung section from a young guinea pig of the type used in this series of experiments. It demonstrates the scarcity of anthracotic pigment phagocytes which are found naturally in the lungs of guinea pigs kept for considerable periods of time in the laboratory pens. × 130.

FIG. 2. A high power lung section of a guinea pig that had received 10 cc. of the standard cell suspension into the jugular vein and was killed 2 days after the injection. Note the numerous carbon-containing mononuclears free in the alveolar spaces. × 700.



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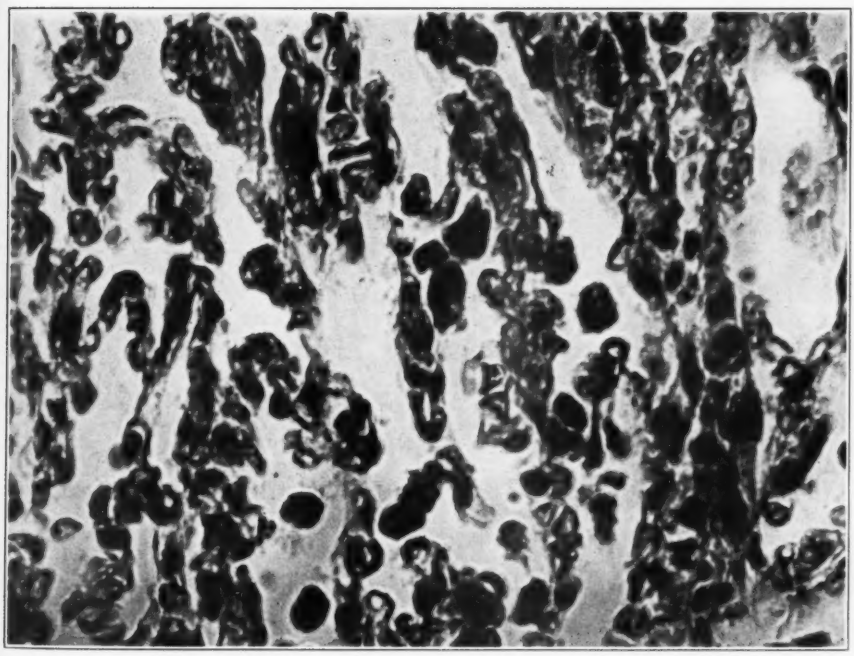

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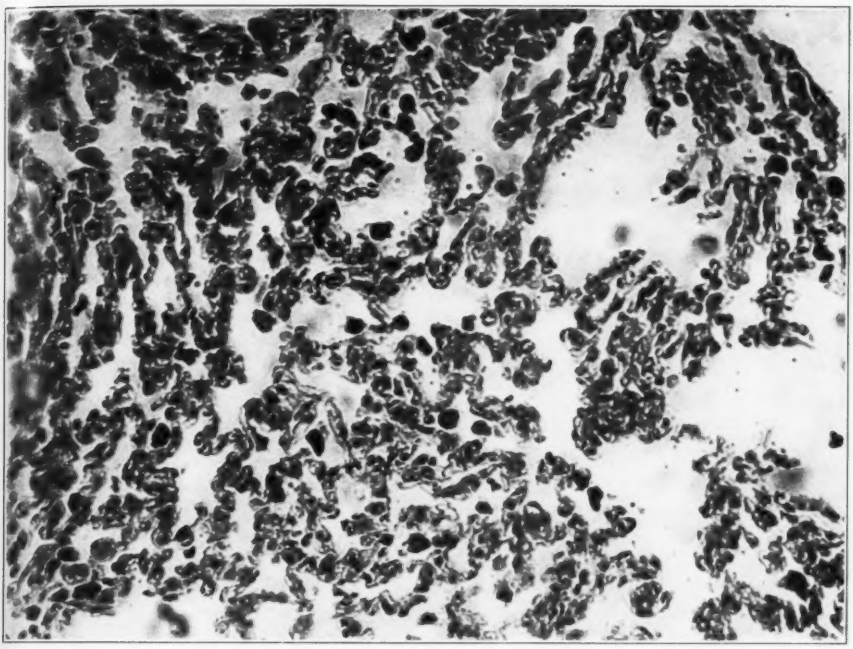
PLATE 95

FIG. 3. A low power photomicrograph of the lung of a guinea pig that had received an intraportal injection of marked cell suspension 5 days before it was killed. It shows not only the many carbon-containing cells which concentrated in the lung, but also demonstrates the fact that the injected cells were not filtered out in the liver. × 200.

FIG. 4. A high power photomicrograph of the lung of a guinea pig into which a suspension of carmine-containing cells had been injected intracardially 2 days before it was killed. Several injected cells are shown which are so closely applied to the alveolar wall that they simulate alveolar epithelium. The pigment as seen in the section was red in color. × 400.



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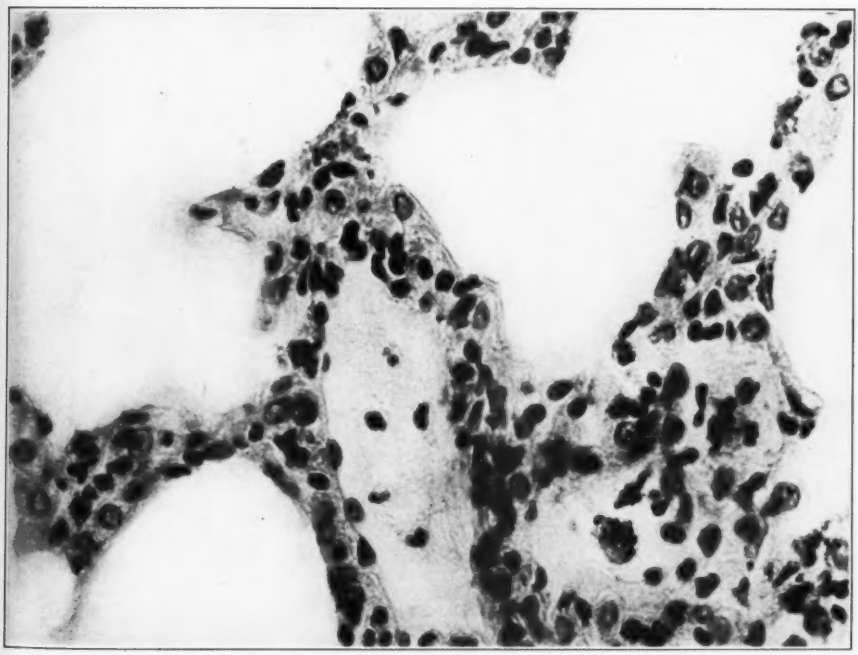

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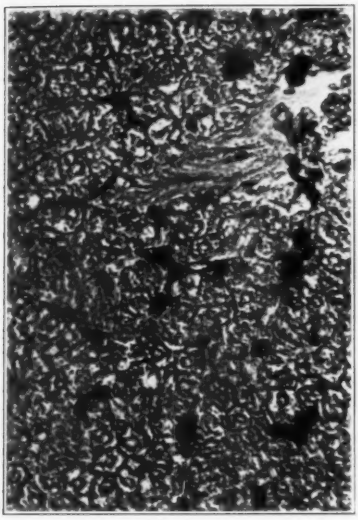
PLATE 96

FIG. 5. The liver of a guinea pig that received dilute India ink intraportally and was killed 3 days later. It demonstrates that when suspensoids, not contained within cells, are injected into the portal vein large quantities of it are retained within the sinusoids, either as embolic masses or within the Kupffer cells. × 200.

FIG. 6. The lung of a guinea pig that received a large amount of India ink suspension intracardially and survived for approximately 24 hours. It demonstrates that even when massive amounts of suspensoid dye are injected at the most favorable locations, no diffusion into the alveolar spaces occurs. × 400.

FIG. 7. The liver of a guinea pig that had received the cell suspension intraportally 3 days before it was killed. Note that only two small particles of carbon are seen in the entire field, demonstrating the fact that the pigment-bearing cells pass through the capillary beds of the liver to gain the lung with very slight destruction. × 200.

FIG. 8. A high power photomicrograph of the lung of a guinea pig that received a suspension of pigment-bearing cells into the jugular vein 24 hours before it was killed. Large, swollen, carbon-containing cells may be seen lining the alveoli in several locations, but of special interest is the cell in the upper alveolus which is in the process of escaping from the capillary to gain the alveolar space. A large portion of this cell is already applied to the alveolar wall but a portion of it may still be seen within the capillary lumen. × 700.



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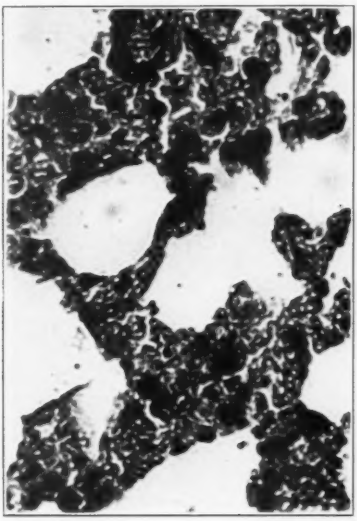

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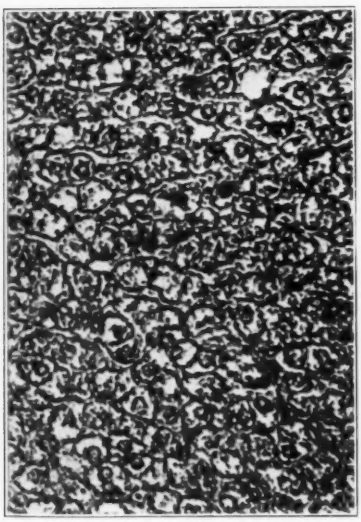

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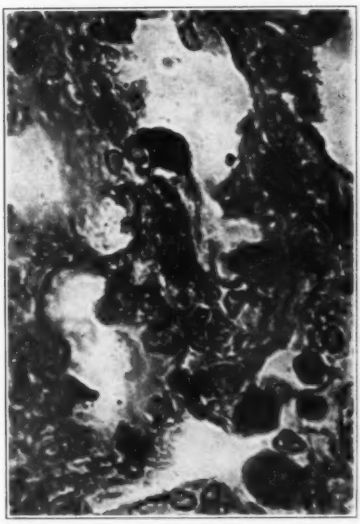

8

CHRONIC PULMONARY ARTERITIS IN SCHISTOSOMIASIS MANSONI ASSOCIATED WITH RIGHT VENTRICULAR HYPERTROPHY *

REPORT OF A CASE

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Schistosomiasis is a rare disease in North America. Cutler,[1] reviewing the literature in 1926, was able to collect 22 cases. No additional cases have been reported in North America since that review. The cases appearing in the United States have been acquired in Africa or the West Indies. In the latter country only schistosomiasis mansoni is endemic.

The life history of the parasites has been studied and reported by various investigators. Most recently Faust, Jones and Hoffman [2] have studied the life cycle and the course of infestation of *Schistosoma mansoni* by the use of experimental mammals, rats, rabbits and monkeys. They find that the lateral-spined eggs, obtained from the stool of patients with the disease, contain ciliated larvae (miracidia). The infective organisms, the cercariae, appear 24 to 35 days later by rupture of the sporocyst. The cercariae, making their way into the animal through the skin, are carried by the blood to the lungs. Thence they migrate passively via the pulmonary venules to the left side of the heart, aorta and mesenteric artery and tend to accumulate in the portal system. From the organs fed by the aorta, they pass again through the lungs and eventually accumulate in the liver. According to Manson,[3] about 6 weeks after penetration of the host the trematodes reach maturity, at which time the females lay their eggs in the portal system.

Dew [4] has studied the lesions produced by the parasite and its ova. The adult female makes its way in the vein against the current. On reaching a point in the vein impeding further progress, the ova are deposited; the spine engages in the wall of the vein, following which

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the ovum is forced through into the perivascular tissues. The ovum is first surrounded by leukocytes, among which the eosinophile is conspicuous. Surrounding the leukocytes there is a reaction of the fixed tissue cells and large and small round mononuclear cells are scattered peripherally. The ovum may be engulfed by phagocytic cells or giant cells may be formed around it. The aggregation of eosinophilic cells, lymphocytes and tissue cells around central giant cells constitutes the bilharzial "tubercle." The phagocytosis of the ovum may go on until it is replaced by fibrous tissue, and finally a fibrous nodule results with fragments of the chitinous eggshell remaining to show its true nature.

In *S. mansoni* infections the severest lesions are found under the mucosa of the colon, rectum, and in the liver. Dew [4] states that in pure *S. mansoni* infections there is an almost complete absence of lung changes, contrasting with the findings in infection with *S. hematobium*. In the latter, pigmentation and patchy fibrosis may be found. However, Hutchison [5] in his discussion of the pathology of schistosoma infections states that "ova have been discovered in the lungs, brain and kidneys." He used material brought from Egypt. Day [6] mentions that bilharzial lesions may be found in the lungs of patients who have died with "bilharzial cirrhosis" caused by the *Schistosoma mansoni*.

Vascular lesions in schistosomiasis were first described in 1905 by Letulle [7] who called attention to lesions of the veins of the intestine in schistosomiasis hematobia. He investigated 1 case of this disease with great thoroughness and described fibrous intimal thickening in the veins of the mucosa, submucosa and muscularis. The thickening was eccentric or concentric and led to considerable diminution in the lumen of the vessel. Nakamura [8] described lesions in the veins in *Schistosomiasis japonica*. These were of various types. There were embolic lesions consisting of ova lodged in the finest portal branches, around which pseudotubercles with foreign body giant cells developed. He also saw thickening of the walls of the larger portal twigs and thrombus formation. Both he and Benda,[9] who studied *S. japonica*, found focal intimal thickening in the branches of the portal vein, which consisted of granulomas comprised chiefly of fibroblasts with few lymphocytes and large giant cells of the Langhans's type enclosing ova or their shells. Benda stated he could find no assertions in the literature concerning vascular lesions

caused by *S. mansoni*. Hutchison [5] asserts that thrombosis of the veins is common, and refers to thrombosis of the femoral, abdominal vena cava and the portal vessels, but does not mention the type of schistosomiasis in which this was observed.

There are two reports of arterial lesions associated with infestation by the trematode. Sorour,[10] from a study of material in Cairo, reported lesions which he considered "exactly similar to the well-known vascular lesions found in syphilitic and arteriosclerotic cases with no bilharzial element." He described lesions in which the presence of the ovum within the lumen excited marked proliferation of the endothelium enclosing the ovum and obliterating the lumen. He noted proliferation of the subendothelial tissue in branches of the pulmonary artery. Bilharzial tubercles were noted within the muscular coat of vessels which revealed an aneurysmal-like vascular projection. He does not state the size or the location of the vessels affected. The type of schistosoma parasite associated with these lesions is not recorded. No illustrations are given.

Bey [11] described 2 cases of dilatation of the pulmonary artery associated with "fibrosis of the lungs and splenomegalic cirrhosis of the liver" in Egyptian peasants heavily infected with bilharzia, both urinary and intestinal. An autopsy performed in 1 case following sudden death revealed the presence of bilharzial lesions in the liver, intestines, bladder and lungs. The lungs are described as "fibrotic, tough and emphysematous." Microscopically there were "advanced peribronchial and perivascular bilharziomata." "The bilharzia ova were deposited in the adventitia of the blood vessels, and in these last, a generalized endarteritis obliterans was quite manifest." The lesions are not illustrated. The pulmonary artery in gross revealed diffuse dilatation and was studded with raised rubbery patches, which microscopically proved to be early atheromatous lesions. In the description of the heart extreme dilatation of the right side was noted and an "embolus" was found filling one of the main branches of the pulmonary artery; the source of the embolus is not mentioned.

Because of the inadequacy of reports in the literature dealing with vascular involvement in schistosomiasis mansoni, and because a cardiac complication has not hitherto been described, it seemed worth while to record the following case.

Report of Case

Clinical History: T. C., a 21 year old Porto Rican female, was admitted to the wards of the Third (New York University) Medical Division of Bellevue Hospital, July 31, 1934, with the chief complaint of shortness of breath and swelling of the legs.

The family history was irrelevant. The patient was born in Porto Rico, where she had lived until the age of 9. For the next 12 years she resided in New York. Since the age of 14 she had had palpitation and shortness of breath on exertion, and during that year she was told by her school physician that she had heart disease. In 1931 precordial pain first appeared. In November, 1931, she entered the Metropolitan Hospital complaining of pain in the right lower abdominal quadrant. The records of that hospital reveal no significant physical findings except for the heart which was described as follows: "The apical impulse was forceful; the point of maximal intensity in the fifth interspace within the midclavicular line. There was an apical systolic thrill and a long musical systolic murmur heard all over the precordium and back; the rhythm was regular." The patient was discharged improved, soon after admission, with the clinical diagnosis of acute salpingitis.

In February, 1932, the patient was readmitted to the Metropolitan Hospital for vaginal discharge and pain in the right upper abdominal quadrant of 2 weeks duration. The findings on this admission were similar to those of the previous admission.

She did not come under medical observation again until her present illness, which began 1 month previous to admission to the Bellevue Hospital. She experienced marked increase in shortness of breath and observed swelling of the legs. Following this, swelling of the abdomen was noted, and dyspnea became so marked that she found it necessary to sleep in the semirecumbent position. For 3 days prior to admission she experienced pains in the precordium and in the right chest.

Physical examination revealed an acutely and chronically ill young negress who exhibited marked dyspnea and orthopnea. Because of her race cyanosis was not evaluated. The pupils were equal, regular and reacted to light. Examination of the ears, nose and throat was negative. The veins of the neck were dilated in the recumbent, semirecumbent and upright positions, pulsated and filled from below. There were dullness, diminished breath sounds and an occasional râle at the right base. The point of maximal intensity of the apical impulse was in the fifth interspace outside the midclavicular line. Systolic and diastolic murmurs were present at the apex; P_2 greater than A_2; rhythm was regular, gallop audible and the rate was 120. The blood pressure was 100/68. The abdomen was distended with fluid; the liver and spleen were not palpated. There was marked pitting edema of the lower extremities extending to the sacrum. The rectal temperature was 101° F.

Laboratory Examination: Red blood cells 3,700,000 per cmm. Hemoglobin 60 per cent. White blood cells 8600 with 76 per cent polymorphonuclear leukocytes, 4 per cent metamyelocytes II, and 20 per cent lymphocytes. Urine was negative but for the presence of a trace of albumin. Blood culture was sterile. Blood chemistry revealed the following values: non-protein nitrogen 47, uric acid 7.5 mg. per 100 cc.

The patient was rapidly digitalized but grew increasingly dyspneic and died 20 hours after admission.

Clinical Diagnoses: *Cardiac: (*a*) unknown (rheumatic type, inactive and active); (*b*) enlarged heart, mitral insufficiency and mitral stenosis; (*c*) sinus tachycardia; (*d*) class III (failure at rest).

Significant Autopsy Findings

Bellevue Hospital autopsy No. 20856, performed on Aug. 1, 1934, 4 hours after death, revealed the body to be that of a young negress about 155 cm. in length and weighing about 120 pounds. There were numerous pinhead macular-papular lesions, dark red in color, over the anterior thoracic region. There was marked edema of the legs, thighs, anterior abdominal wall and sacrum. The nailbeds were dusky blue, and there was no clubbing. There were wedge-shaped hemorrhages in the sclera of both eyes on either side of the pupil.

On section the subcutaneous fat was moderate in amount, the muscle tissues well developed and deep red in color. There were about 2000 cc. of clear amber fluid in the peritoneal cavity. The peritoneum was smooth and glistening but the subserosal tissues were markedly edematous.

On opening the chest the precordium was seen to be markedly enlarged, chiefly to the right, compressing and displacing the right lung. Both pleural cavities were dry and there were no adhesions between the lungs and the chest wall.

Heart: On opening the pericardial sac about 300 cc. of a clear, straw-colored fluid were encountered. There were no adhesions between the visceral and parietal layers of the pericardium. The anterior aspect of the heart was occupied chiefly by the right ventricle. The right auricle was found to be dilated. The orifice and the leaflets of the tricuspid valve were normal. The right ventricle was found to be dilated and its walls hypertrophied. The maximum thickness of the wall of the right ventricle was 8 mm. (Fig. 1). The pulmonary artery was dilated, measuring 7 cm., while the aortic ring measured only 5 cm. The pulmonic cusps were thin, transparent and freely movable. A few circumscribed, irregular, raised yellowish plaques were visible in the various sized branches of the pulmonary artery. The left side of the heart appeared dwarfed by comparison with the

* Diagnosis conforms to nomenclature of American Heart Association.

right. The maximum width of the wall of the left ventricle was 10 mm. The mitral and aortic valves were normal. The aorta and coronary vessels presented no notable characteristics. The heart weighed 360 gm.

Lungs: Both lower lobes were distinctly heavier than the upper and middle lobes and of a deep red color, contrasting with the pinkish gray color of the latter. On section a red frothy fluid exuded from the lower lobes whereas the upper lobes were but slightly moist. The small arteries appeared to project from the surface and felt thickened. The trachea and bronchi contained a slight amount of mucoid material.

Liver: The liver appeared small, weighing 960 gm. Its surface was nodular, the nodules varying in diameter from 2 to 10 mm. The color was a yellowish brown with irregular patches of purple. The organ was of increased firmness and cut with increased resistance. The essential pattern of the cut surface was that of a mosaic, the units of which were round or oval in outline and varied from 1 to 2 cm. in diameter (Fig. 2). The periphery of these units was formed by an irregular dark red border 1 to 3 mm. in width. In the center was a vessel cut in cross-section, 2 to 3 mm. in diameter and surrounded by a small amount of connective tissue. The intervening tissue revealed no distinctive features. The portal vein was empty and its walls appeared normal. No adult worms were found.*

The gall-bladder and biliary passages were normal.

Spleen: The organ was about thrice its normal size, purplish red in color with a thin smooth capsule. On section it presented a smooth surface, dark homogeneously red and firm. The follicular markings were not visible. The splenic vein was normal.

Gastro-Intestinal Tract: In the lower third of the esophagus several dilated veins were readily made out and two bleeding points about 4 cm. above the cardia were seen. The stomach was moderately dilated and contained about a half liter of coffee-ground material and several small blood clots. Thin, blackish brown material was also seen in the small intestine. The mucosa and intestinal wall, including that of the rectum, appeared normal. The hemorrhoidal veins were not opened.*

The pancreas, adrenals, kidneys, ureters and bladder were normal.

* On gross examination the diagnosis of schistosomiasis was not suspected.

Genitalia: There were two hemorrhagic cysts on the surface of both ovaries, each about the size of a pea. The tubes and uterus were normal.

Head: Not done.

Microscopic Examination

Sections were fixed in formalin, stained with hematoxylin and eosin, Weigert's elastic tissue method, Van Gieson, and occasional sections were stained with the silver impregnation method of Foot and Foot.

The diagnosis of schistosomiasis was established by the presence of ova of this trematode in many organs. They were found in large numbers in fixed tissue sections in the lungs, myocardium, liver, pancreas and kidney. The most typical ovum seen in fixed tissue sections appears as an oval eosinophilic mass 35 to 70 μ, containing irregularly scattered, coarse, basophilic chromatinic material, and separated by a clear space from the chitinous shell. The latter is irregularly wavy, continuous or interrupted, single or appearing reduplicated, and has a clear, refractile, straw-colored appearance (Fig. 3). Occasionally a laterally placed spine is seen. The lesions provoked by the presence of these ova are to be described in the individual organs in which they occurred.

Liver: In sections the structural unit appears to be that consisting of a centrally placed, massively thickened portal radicle surrounded by liver lobules and outlined peripherally by an area of hemorrhage and necrosis of the liver trabeculae. The central area consists of rather dense collagen which displays radiating prolongations into the surrounding tissue. The periportal tissue is markedly increased in amount. There are several thin-walled veins and small arteries cut in cross-section, the walls of the latter being somewhat thickened. Medium size bile ducts are included. The connective tissue reveals foci of cells. Many such foci consist of a central core of an ovum surrounded by lymphocytes, eosinophilic leukocytes and plasma cells. Other foci show dense accumulation of lymphocytes with few eosinophiles and plasma cells and no ova. The inflammatory reaction is limited, chiefly, to the areas surrounding the ova; there is no reaction in the tissue surrounding the vessels or ducts. Ova are numerous, 1 to 2 are present in each medium power field.

The surrounding parenchyma has retained its identifying architecture, with liver trabeculae and portal radicles, and occasional central veins. The trabeculae are for the most part well preserved in outline, but the cells stain faintly with eosin. Scattered irregularly are large areas of very poorly staining cords showing granular disintegration of the cytoplasm with disappearance of the nuclei and at times complete disintegration of the cords. The Kupffer cells contain brown pigment which does not give the iron reaction. The portal radicles encountered in these areas reveal no notable change other than the presence of a degenerated ovum and inflammatory cells. The more peripheral hemorrhagic zone consists of broad, irregular areas of intense hemorrhage with obliteration of any recognizable liver trabeculae. Between these areas of hemorrhage are those in which hemorrhage is absent, liver trabeculae have disappeared and there remains but the reticular framework of the organ with many proliferating bile ducts.

Spleen: There is marked diffuse congestion of the pulp and a fine increase in the connective tissue framework. There are numerous polymorphonuclear leukocytes and a few eosinophiles visible in the pulp. No ova are seen.

Pancreas: Foci of ova surrounded by inflammatory cells similar to those in the liver are seen. In some areas the interlobular connective tissue is diffusely infiltrated by cells among which the eosinophilic leukocyte predominates. The ova are found intralobularly as well as in the interlobular tissue. There are no areas of necrosis, no increase in the stroma of the gland, and the vessels appear normal.

Kidney: Many ova are seen in the cortex, some of which are recognizable only by the chitinous shell. They are surrounded by numerous lymphocytes and eosinophilic polymorphonuclear cells. They occupy an interstitial position without any particular relation to the blood vessels; the latter appear normal. The glomeruli and tubules are normal.

Myocardium (Right Ventricle): There is evidence of hypertrophy of the muscle nuclei and fibers and focal interstitial fibrosis. An occasional ovum surrounded by a few inflammatory cells is seen. The left ventricle reveals the presence of a few ova and inflammatory cells. The blood vessels appear normal.

Aorta: The vessel appears normal.

Pulmonary Artery: The intima is slightly thickened and presents atheromatous plaques.

Lung: The pulmonary parenchyma appears for the most part normal. There are irregular areas of marked congestion, some of focal alveolar hemorrhage and patchy collapse. The striking finding is that of granulomatous lesions within or related to the blood vessels. Some of these granulomas appear clearly to be in the periadventitial connective tissue and the vessel may appear normal. The granuloma consists of one or more ova, which may contain a foreign body giant cell. These ova are surrounded by young fibrillar tissue (argentophile fibers) in which are spindle and oval fibroblasts, lymphocytes and eosinophiles, and numerous, small endothelial-lined channels. But more frequent than these extravascular lesions are those that appear more intimately related to the blood vessels. Figure 4*b* illustrates one such lesion. It appears as a vascularized granuloma in the interstitial tissue related to a bronchus. In the hematoxylin and eosin sections it is chiefly its relation to a bronchus which suggests that it is an artery. Examination of the Weigert elastic sections strengthens this impression, for a wavy elastic membrane outlines the structure peripherally. Indeed, it is possible to see fragments of internal and external elastica with intervening muscle in the periphery of this granuloma. But in order to ascertain with greater certainty the relation of such structures to the pulmonary arteries, serial sections were studied. Figure 4*a* shows a small artery with thickened intima, measuring 200 μ in diameter, cut in somewhat longitudinal section. Figure 4*b*, which is 55 μ distal, reveals the same vessel, considerably dilated, the lumen obliterated and replaced by an ovum surrounded by young granulation tissue with numerous endothelial-lined channels. The media is represented by internal and external elastica with intervening muscle in about a fourth of its circumference; fragmented elastic fibers are seen peripherally for part of its circumference. A mantle of inflammatory cells surrounds the structure and infiltrates the wall of the adjacent bronchus. The cells are chiefly of the lymphocytic type; few eosinophiles are seen. Figure 4*c*, 110 μ distal to the preceding section, is apparently distal to the granulomatous lesion; the lumen of the artery is patent, its wall intact; the diameter of the vessel is half that in the preceding section.

Many small arteries are seen showing marked concentric intimal thickening with diminution of the lumen. The intimal thickening

consists of young connective tissue, avascular and containing but few fibroblasts. Medial hypertrophy appears to be present. Several such vessels were traced serially and their association with a schistosomal lesion was demonstrated. Figure 5*a* illustrates another lesion of this type in a small artery 250 μ in diameter. As this vessel is followed distally, a granuloma can be seen to form between the external elastic membrane and the adventitia. The elastica, in further sections, becomes reduplicated, then disrupted and the vessel assumes an aneurysmal dilatation with the lumen almost completely occluded by proliferating tissue continuous with the intramural granuloma. Figures 5*b* and 5*c*, 195 μ distal to the preceding section, illustrate these changes. Comparison of Figures 4*a*, 4*b* and 4*c* reveals the striking dilatation of the vessel in its distal course. Three ova are to be found in the course of this vessel, two of which occupy an intramural position.

Final Pathological Diagnoses: Lungs: Chronic pulmonary arteritis (*Schistosoma mansoni*), intimal hyperplasia and focal medial hypertrophy, congestion, focal alveolar hemorrhage, patchy collapse. *Heart:* hypertrophy and dilatation, chiefly right ventricular, miliary schistosomal granulomas, dilatation and atheroma of the pulmonary artery. *Liver:* chronic diffuse granulomatous hepatitis (*S. mansoni*), chronic passive congestion, focal hemorrhage, focal necrosis. *Spleen:* chronic passive congestion. *Pancreas:* miliary schistosomal granulomas. *Kidneys:* miliary schistosomal granulomas.

Discussion

While there are many features for discussion in this case, including the widespread dissemination of the ova and the peculiar hepatic lesion, we shall confine ourselves to a consideration of the findings in the pulmonary vessels. The demonstration of ova in constant intimate relation to the vascular lesions in the lungs leaves no doubt as to the parasitic etiology. The presence of the ovum within the lumen or the wall of the vessel has been followed by complex changes leading to the obliteration of the lumen by a richly vascularized tissue. The absence of early lesions in the pulmonary vessels does not permit reconstruction of these lesions from their inception. The rich vascularization of the tissue surrounding the ovum and occluding the lumen might suggest this lesion to be the result of thrombosis with

organization and canalization. But the severe medial changes certainly must be attributed to an injury of a different nature. The absence of collagen and the presence of proliferating endothelium, forming new channels, suggest that these lesions are still young and blood pigment should be present nearby if thrombosis had occurred. Moreover, the same proliferation of endothelial-lined channels is seen in extravascular granulomas in the lung. It appears more likely that the intimal and endothelial proliferation represents a response to the presence of the foreign body.

The medial injury might be effected from within by an expanding proliferative lesion, or from without by the presence of an inflammatory and proliferative reaction provoked by the intramural ova. It appears likely that the intimal thickening and medial hypertrophy of the small arteries proximal to the granulomatous lesion represents a response to the obstruction. The atheroma of the pulmonary artery similarly is to be interpreted as a response to the increased intravascular tension which had its origin in the obstructive arterial lesion.

Thus, the vascular lesions may be separated into two groups: those directly attributable to the ova, and those secondary to the increase in intra-arterial tension. The former may be considered a specific arteritis, characterized by an obliterating endarterial proliferation with marked injury to the media leading to dilatation of the vessel. The latter may be considered as representing a nonspecific vascular change of an arteriosclerotic and atheromatous nature.

The presence of hypertrophy of the right ventricle and evidence of congestive heart failure are obviously related to the lesions of the small pulmonary arteries.

The case presented thus represents one in which the cardiac changes have occurred secondary to pulmonary arterial disease. In this respect it is to be compared with those cases of pulmonary heart disease associated with arterial and arteriolar lesions of the lungs and described under the terms of primary sclerosis of the pulmonary artery (Bacon and Apfelbach [12]), die primäre Pulmonalsklerose (Steinberg,[13] Höra [14]), and Arteriopathia pulmonalis (Bredt [15]). There is little known concerning the etiology of these cases. Arrillaga [16] believed some cases to be due to syphilis. Steinberg [13] described a case in which the vascular lesions were interpreted as

pulmonary arteriolosclerosis secondary to "essential pulmonary hypertension." Bacon and Apfelbach [12] reported a case in which the symptoms appeared after influenza, and they suggested a relation of the pulmonary vascular lesions to the latter disease. Rössle [17] considered the cases described by Bredt as representing a non-specific allergic arteritis related to rheumatic fever and assigns to them the term "rheumatoid vascular disease." Höra [14] interpreted the lesions in his case as evidence of chronic sepsis. In all these cases morphological comparisons have served as the basis for the interpretation of their etiology. In our case of schistosomiasis mansoni the presence of the etiological agent in the inflammatory lesions of the smaller pulmonary arteries has been demonstrated.

Summary

A case of schistosomiasis mansoni in a young Porto Rican dying in congestive heart failure has been recorded. Hypertrophy of the right ventricle, dilatation of the pulmonary artery and lesions of the small pulmonary arteries have been described, and it has been demonstrated that the latter represent a form of specific arterial disease caused by the presence of ova of *Schistosoma mansoni*.

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DESCRIPTION OF PLATES

PLATE 97

FIG. 1. An anterior view of the opened heart revealing the dilated pulmonary conus and marked hypertrophy of the right ventricular myocardium.

FIG. 2. A view of the cut surface of the liver showing the peculiar lobulations and zones of hemorrhage.

FIG. 3. Photomicrograph of a section of the pancreas showing two ova in a granulomatous nodule. Hematoxylin and eosin stain. × 350.

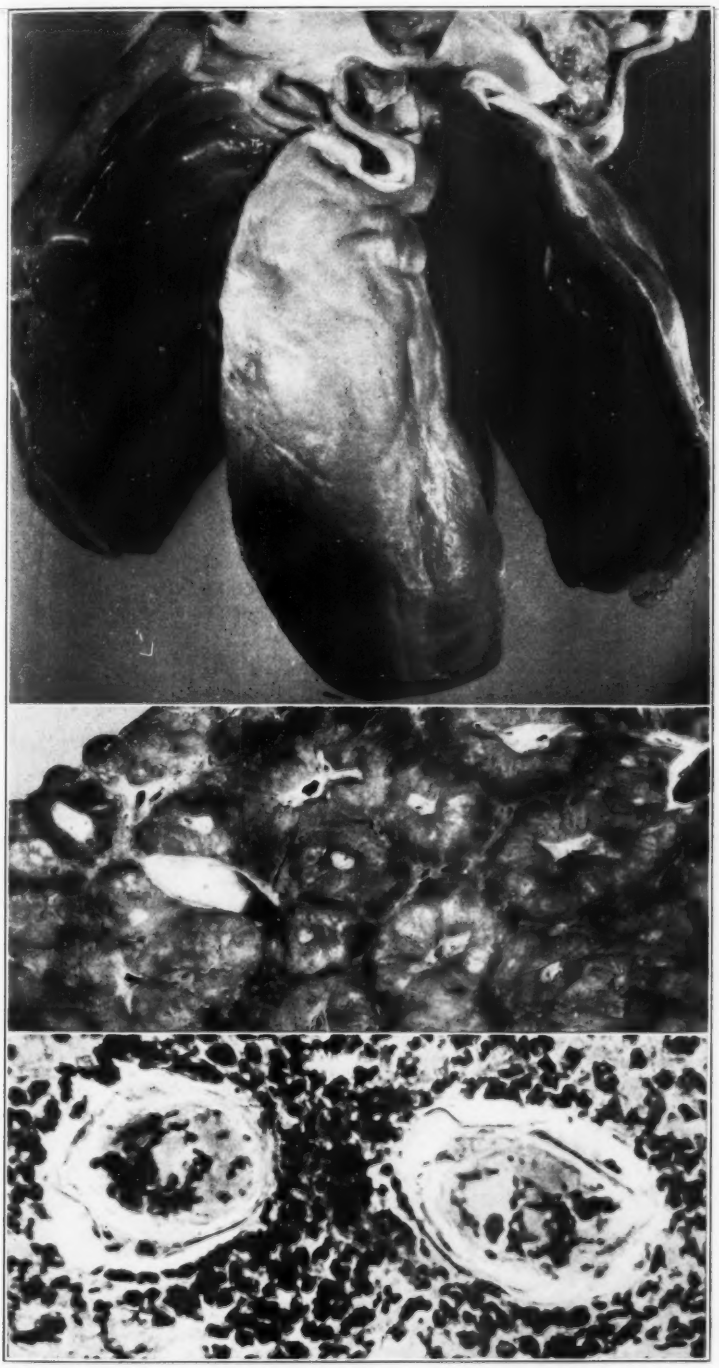

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PLATE 98

FIG. 4*a*. Photomicrograph of a section of the lung showing a small pulmonary artery with thickened intima and beneath it a partially collapsed normal bronchus. Hematoxylin and eosin stain. × 110.

FIG. 4*b*. Same vessel 55 μ distal to preceding section. Note the presence of an ovum within the granulomatous arterial lesion, and a segment of the media with internal and external elastic membranes. Weigert's elastic stain. × 140.

FIG. 4*c*. Same vessel 110 μ distal to preceding section. The section is distal to the granulomatous lesion and dilatation is no longer evident. Weigert's elastic stain. × 95.

FIG. 5*a*. Photomicrograph of a section of another small pulmonary artery revealing marked intimal thickening and medial hypertrophy. A portion of the wall of the adjacent bronchus can be seen at the bottom of this and succeeding photographs. Weigert's elastic stain. × 180.

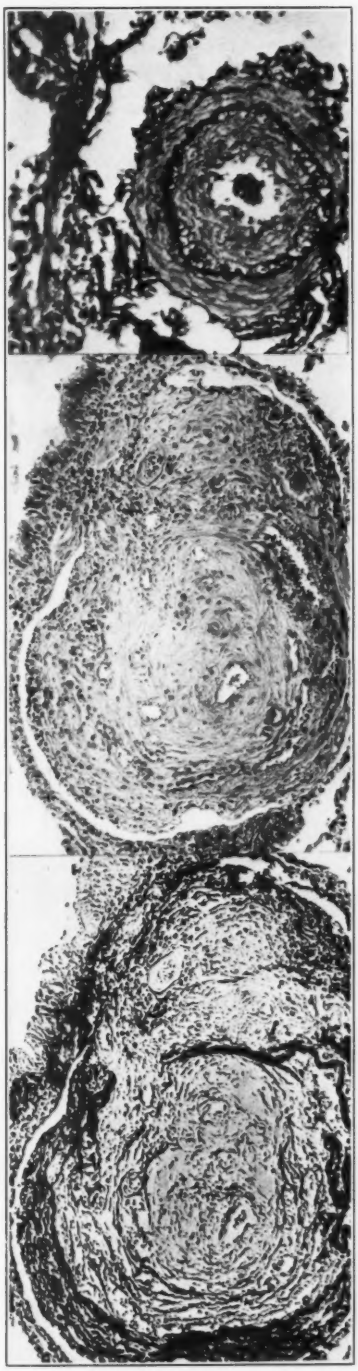
FIG. 5*b*. Same vessel, 195 μ distal to the preceding section, revealing marked dilatation, replacement of the walls and occlusion of the lumen by vascularized granulomatous tissue containing several ova. Hematoxylin and eosin stain. × 140.

FIG. 5*c*. Same section stained with Weigert's elastic tissue stain. Note the disruption of the elastic membrane. × 140.

4a

4b

4c



5a

5b

5c

Clark and Graef

Pulmonary Arteritis in Schistosomiasis Mansoni

EARLY CARDIAC INFARCTION CAUSED BY AN EMBOLUS OF CASEOUS TUBERCULOUS MATERIAL *

REPORT OF A CASE

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Gouley *et al.*,[1] state in their recent report on tuberculosis of the myocardium that "while tuberculous pericarditis is not uncommon, tuberculous involvement of the myocardium is decidedly rare." The author presents in this connection a tuberculous involvement of the heart which he has been unable to find referred to in the literature, namely, infarction of the heart muscle due to an embolus of caseous tuberculous material.

The case was that of a white male aged 40 years whose death was entirely unexpected. The man had been ill for over 6 months but was not considered ill enough to be confined to bed during this time. He was an ambulatory patient up to the day of his death. A diagnosis of pulmonary tuberculosis had been made but this condition was not considered of sufficient severity to account for the sudden death. Because of the circumstances of death a medicolegal autopsy was requested.

The autopsy revealed extensive bilateral pulmonary tuberculosis with a single small cavity present in the upper right lobe. There was also a generalized miliary tuberculosis. The finding of main interest was the presence of a recent infarction of the lateral and upper portion of the left ventricle. The infarcted area measured 3 cm. in length, 1.5 cm. in breadth and extended to within a short distance of the endocardium. In the gross it presented the usual appearance of an infarct of not more than a few days duration. The epicardial vessels over the area were congested and there was a zone of congestion about the gray, swollen, infarcted muscle tissue.

The heart presented no pathological lesions other than the infarct. The valves and endocardium appeared normal. The coronary vessels, as far as could be determined on gross dissection, showed no

* Received for publication March 15, 1935.

atherosclerosis and no gross evidence of thrombosis or embolism. The pericardial cavity contained about 50 cc. of a light yellowish, slightly turbid fluid.

The nature of the infarction was not suspected until a microscopic study was made. The infarcted muscle cells were disintegrating. There was considerable edema and abundant cellular infiltration, especially around the periphery of the lesion. The cells were largely monocytes and lymphocytes with, in places, a generous sprinkling of neutrophiles. There was no evidence of tubercle formation or Langhans's giant cells. In other words, the histopathological picture of the infarcted heart muscle was that of a non-infectious lesion.

In the epicardium a small branch of the coronary artery lying over the infarcted area was found to be plugged by a bit of caseous material in which tubercle bacilli were easily found. Even here there was no evidence of tubercles, giant cells or even acute inflammation. The wall of the artery appeared to be normal. Very careful search failed to reveal any tubercle bacilli within the area of infarction.

We have here, then, in an individual with generalized miliary tuberculosis, an early, acute cardiac infarct caused by a bit of caseous material from a pulmonary tuberculous lesion. As a tuberculous embolus it entered the circulation in the pulmonary vein and lodged in a small branch of the left coronary artery.

Comment

It is becoming generally recognized that hematogenous dissemination of tubercle bacilli is the predominant method by which tuberculous foci are established outside of the respiratory and gastrointestinal tissues. This of course excepts the lymph nodes, such as the cervical, peribronchial and mesenteric, which usually become involved through lymph drainage from foci of infection in the tonsil, lung or intestine.

From the literature it would seem that there is a consensus of opinion that tuberculous involvement of the heart and its membranes occurs through a reversal of lymph flow from mediastinal tuberculous nodes. Thus, Gouley *et al.*,[1] give the impression that 5 out of their 6 cases of myocardial tuberculosis are thus caused. Kast [2] reports a case in which he considers the heart involvement was brought about through the rupture of a caseous node into the

pericardium. It appears that the direct extension, by rupture, of tuberculous disease into the pericardial sac might account for an occasional case of tuberculous pericarditis. It does not appear logical, however, that reversal of lymph flow from tuberculous lymph nodes in the mediastinum would account for the majority of tuberculous involvements of the epicardium and myocardium.

Perhaps one of the reasons why the hematogenous nature of tuberculous foci in the heart or the pericardium is not appreciated is that the bit of tuberculous material is so small that it is extremely difficult to identify within the lumen of a capillary around which develops the larger lesion. Another reason for failure to appreciate the hematogenous nature of the lesion is that by the time the case has come to autopsy the disease process has been in existence so long that it is impossible to determine the site and nature of the original lesion.

The cardiac involvement reported in this article is of real significance relative to hematogenous dissemination of tuberculous material. First, the lesion is of such recent occurrence that the nature and location of the tuberculous material can definitely be determined. Second, the caseous embolus, having demonstrable tubercle bacilli, was within a coronary artery. And third, the embolus was of sufficient size to cause a typical infarct of considerable size in the left ventricular wall.

SUMMARY

A case of cardiac infarction due to a caseous tuberculous embolus within a branch of the left coronary artery is reported.

NOTE: The material for this report was made available through Dr. A. W. Johnson, Coroner for Saratoga County, N. Y.

REFERENCES

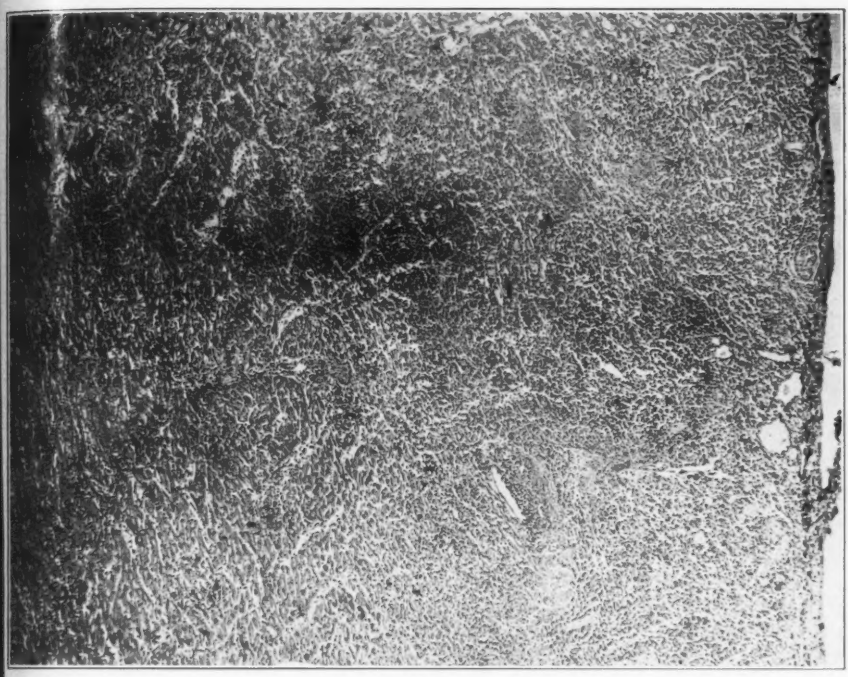
1. Gouley, B. A., Bellet, S., and McMillan, T. M. Tuberculosis of the myocardium; report of six cases, with observations on involvement of coronary arteries. *Arch. Int. Med.*, 1933, **51**, 244–263.
2. Kast, A. Ueber eitrige Pericarditis bei Tuberculose der Mediastinaldrüsen. *Virchows Arch. f. path. Anat.*, 1884, **96**, 489–500.

DESCRIPTION OF PLATE

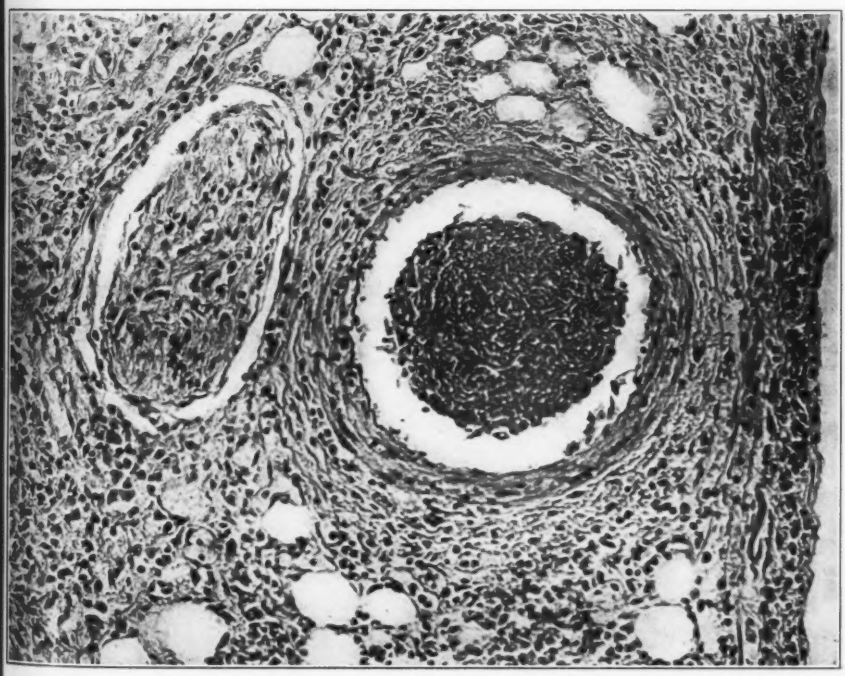
PLATE 99

FIG. 1. An area of the infarcted heart muscle showing extensive cellular infiltration. × 50.

FIG. 2. Section showing caseous embolus within the coronary artery. × 300.



1



2

Medlar Cardiac Infarction caused by Tuberculous Material